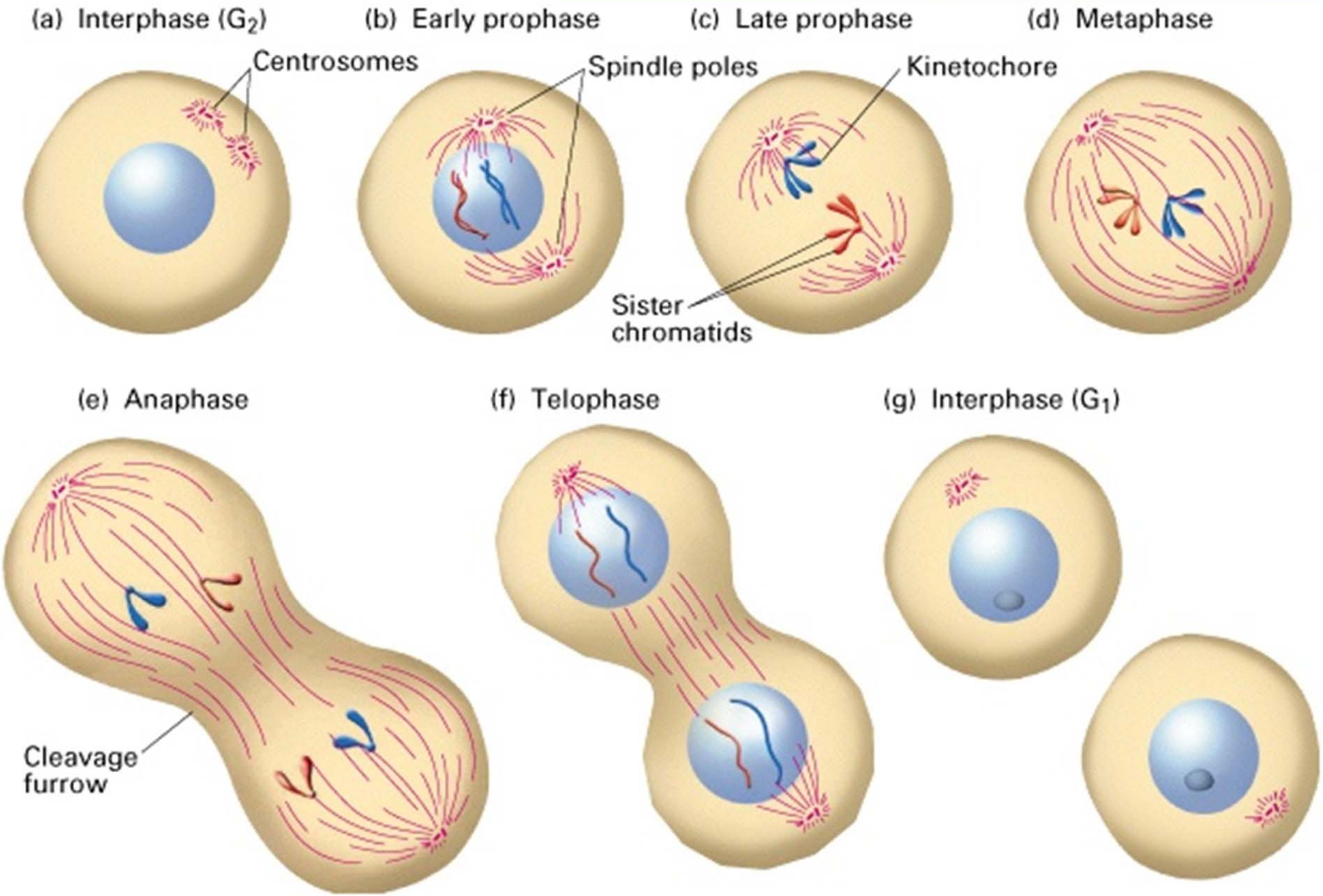
Buněčný cyklus - principy regulace buněčného růstu a buněčného dělění

Mitóza



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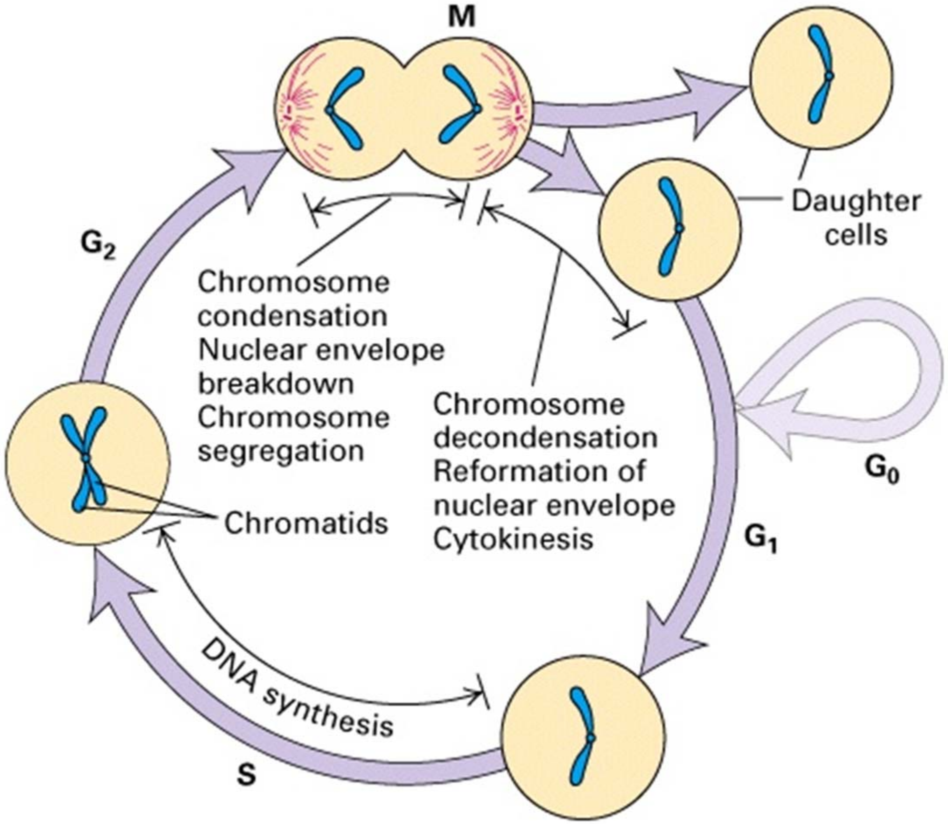
Buněčný cyklus



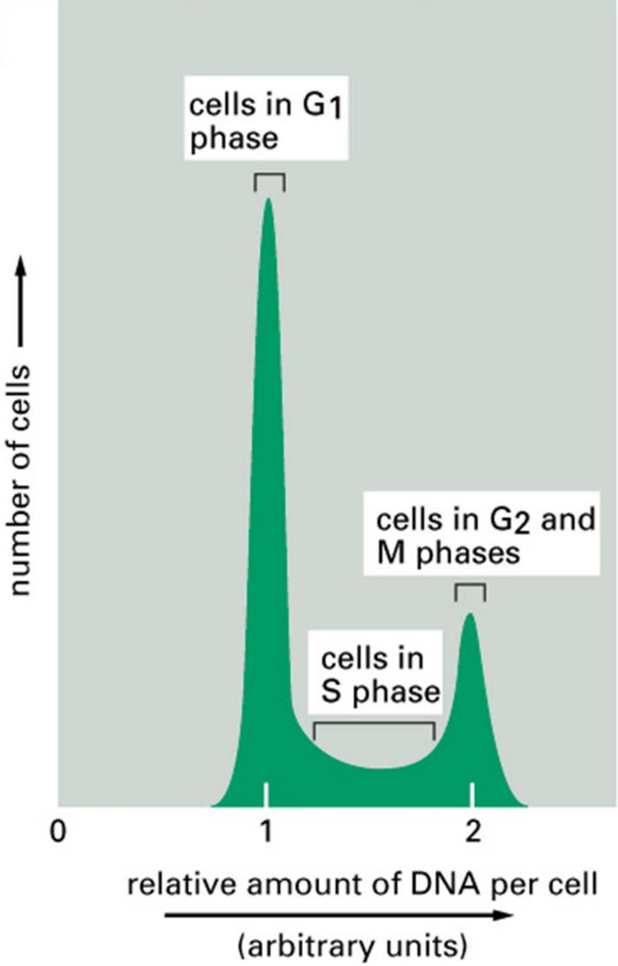
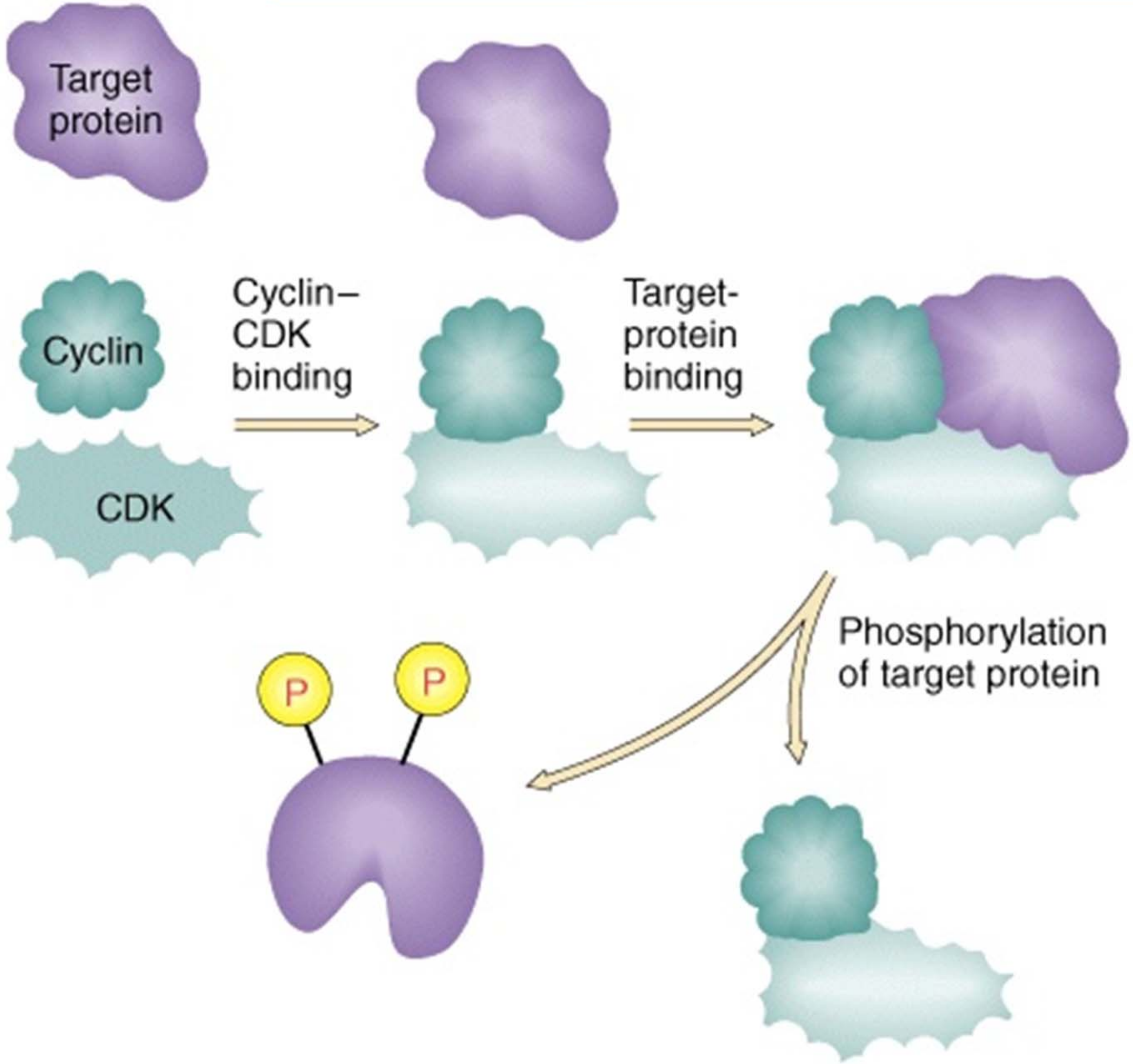


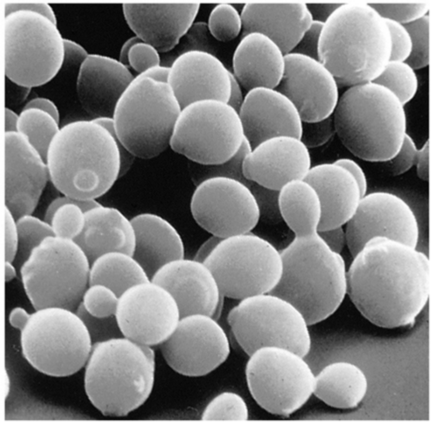
Figure 17–12. Molecular Biology of the Cell, 4th Edition.

Kinázy závislé na cyklinech kontrolují buněčný cyklus

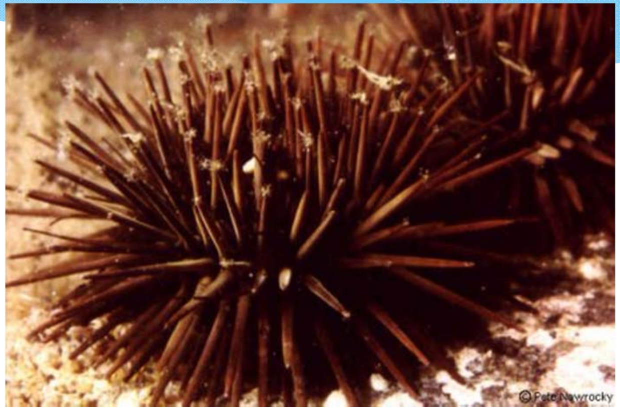


Kontrola vstupu do mitózy
- výsledek využití různých buněčných modelů

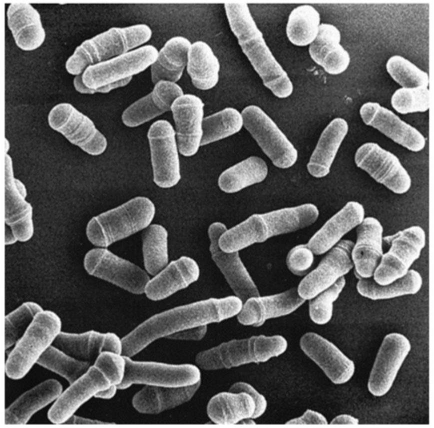
Experimental Systems Important for Cell Cycle Studies



Saccharomyces cerevisiae



Arbacia punctulata



Schizosaccharomyces pombe

Xenopus laevis

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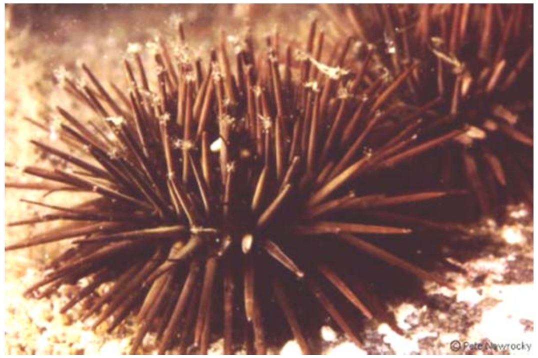
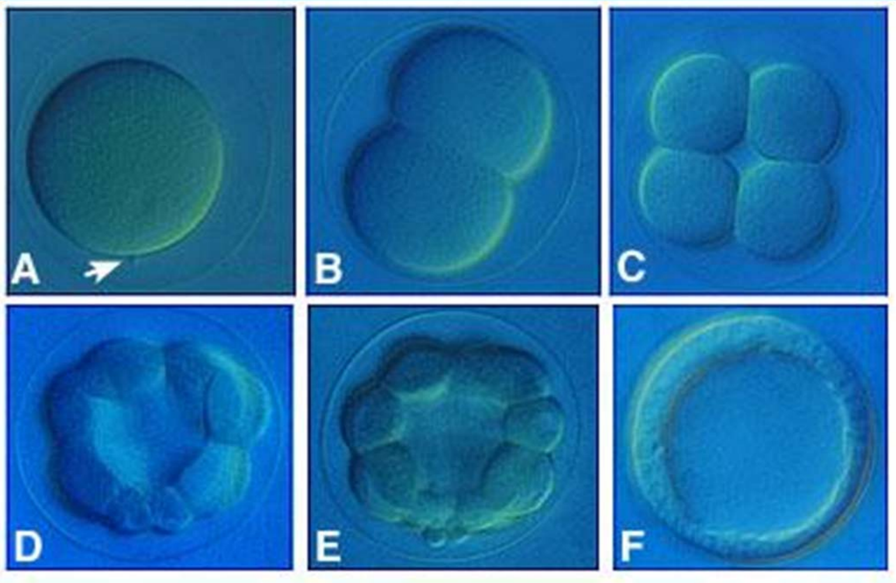
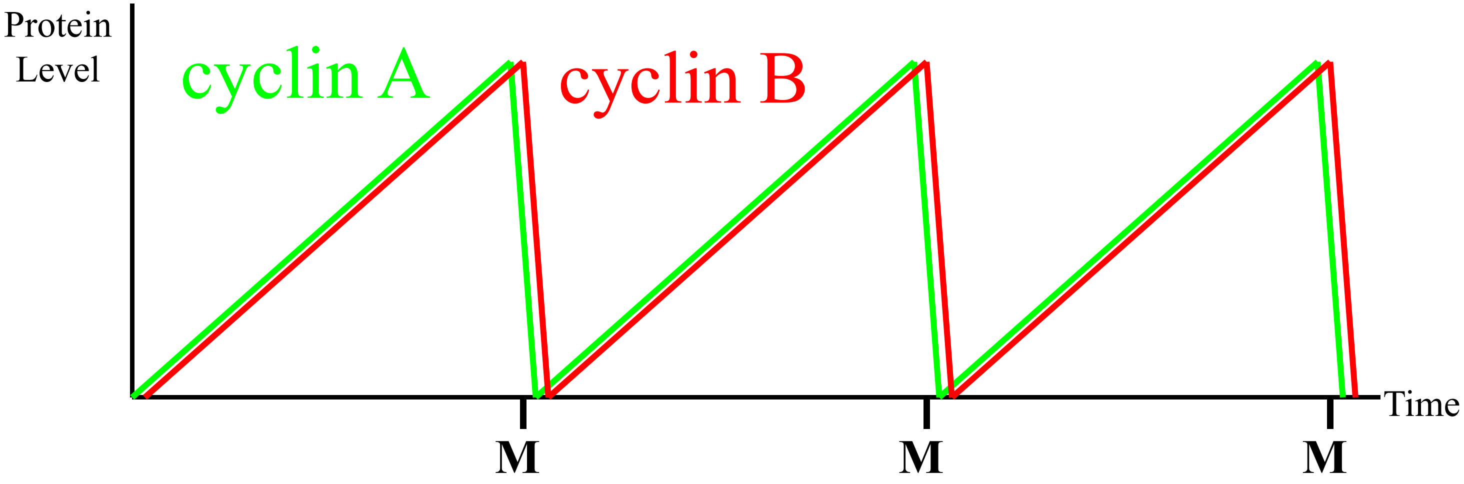
Cyclin was Discovered in Sea Urchin Embryos



can stimulate to

lay lots of eggs





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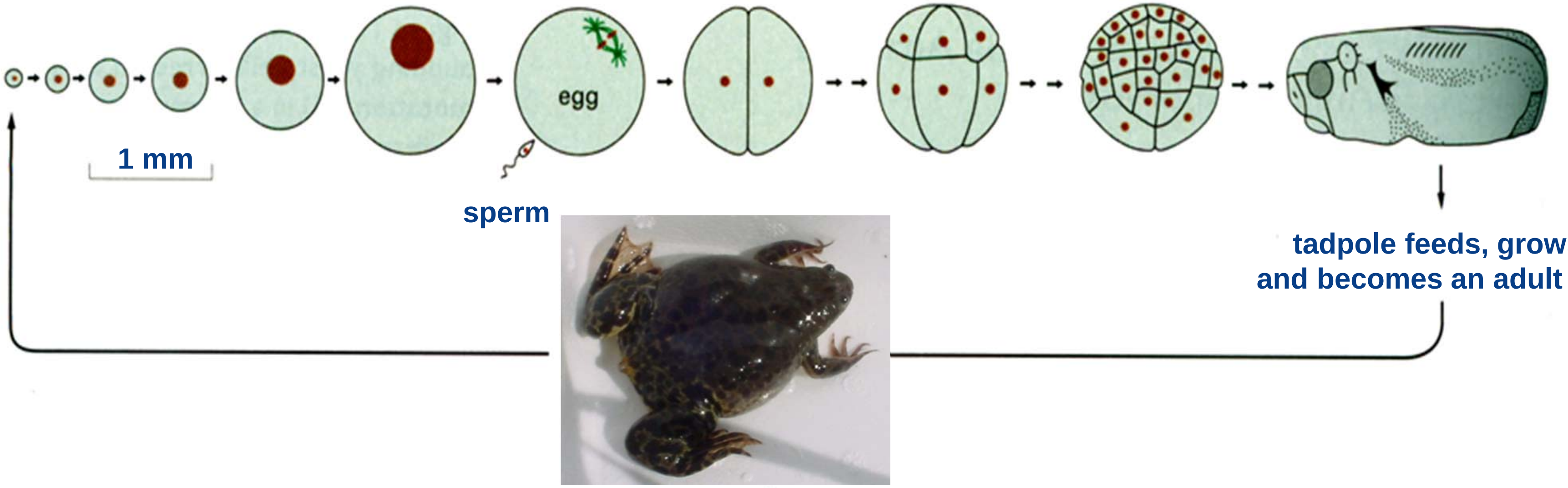
Frog life cycle



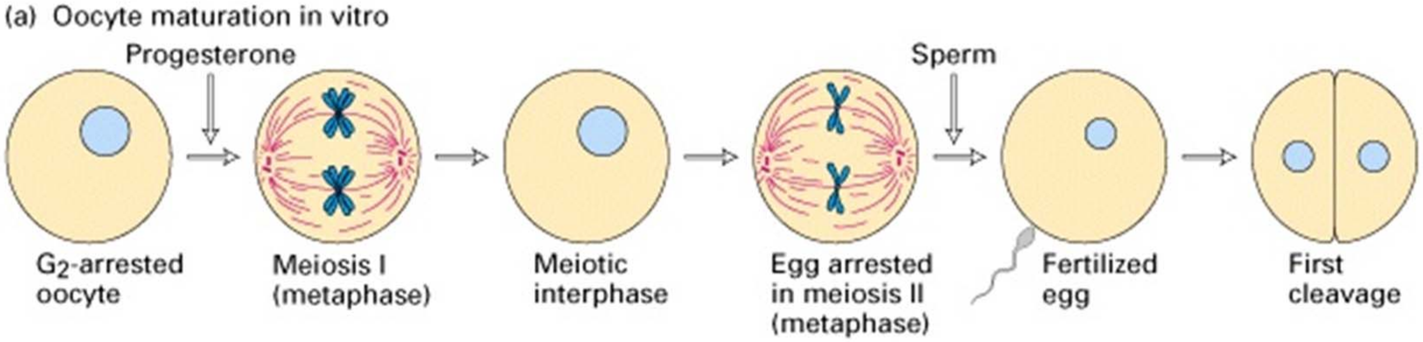
OOCYTE GROWS WITHOUT DIVIDING (MONTHS)

FERTILIZATION

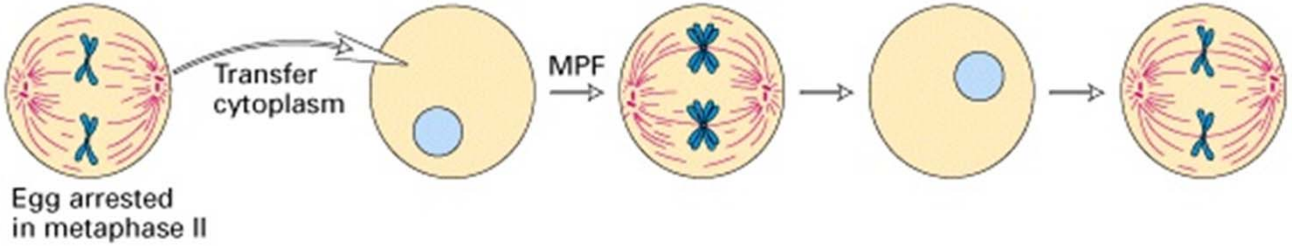
FERTILIZED EGG DIVIDES WITHOUT GROWING (HOURS)

tadpole feeds, grows and becomes an adult frog

The Maturation of Frog Eggs

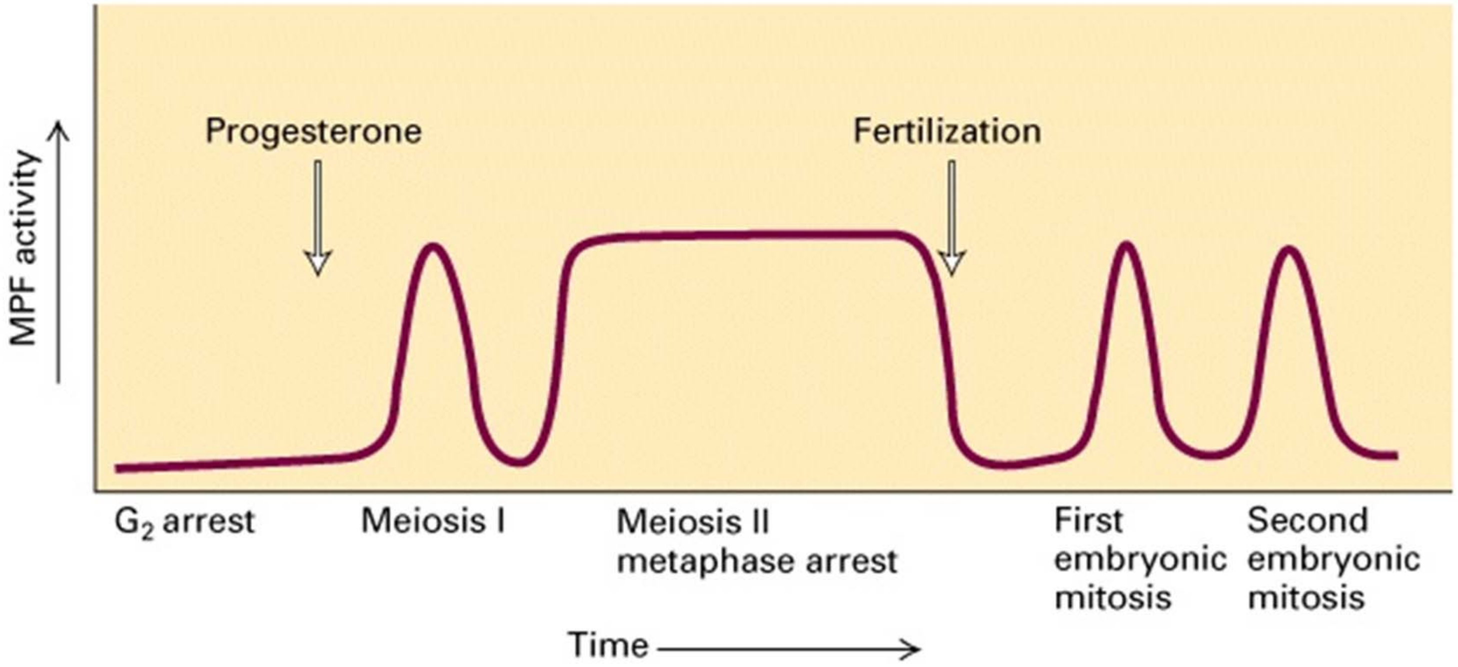


An Assay for Maturation Promoting Factor (MPF)

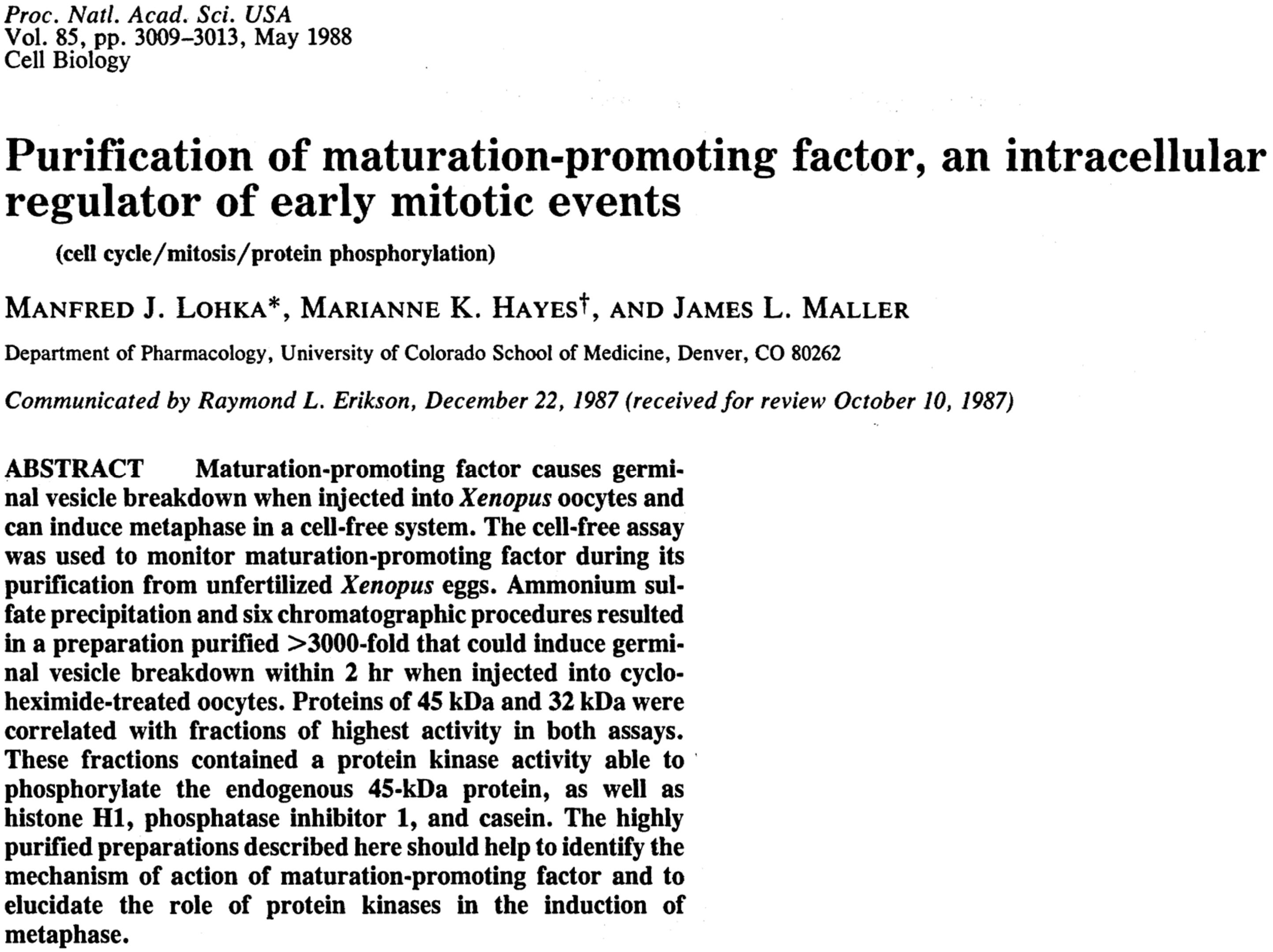


Yoshio Masui, 1971

MPF Activity Peaks Before Each Cell Division



Moreover, MPF has kinase activity



Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 3009–3013, May 1988
Cell Biology

Purification of maturation-promoting factor, an intracellular regulator of early mitotic events

(cell cycle/mitosis/protein phosphorylation)

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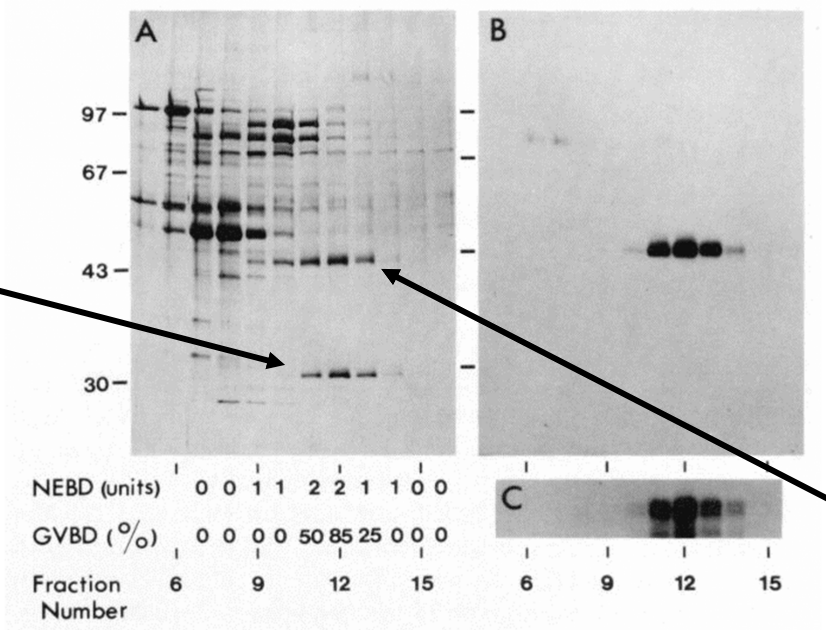
Department of Pharmacology, University of Colorado School of Medicine, Denver, CO 80262

Communicated by Raymond L. Erikson, December 22, 1987 (received for review October 10, 1987)

ABSTRACT Maturation-promoting factor causes germinal vesicle breakdown when injected into *Xenopus* oocytes and can induce metaphase in a cell-free system. The cell-free assay was used to monitor maturation-promoting factor during its purification from unfertilized *Xenopus* eggs. Ammonium sulfate precipitation and six chromatographic procedures resulted in a preparation purified >3000-fold that could induce germinal vesicle breakdown within 2 hr when injected into cycloheximide-treated oocytes. Proteins of 45 kDa and 32 kDa were correlated with fractions of highest activity in both assays. These fractions contained a protein kinase activity able to phosphorylate the endogenous 45-kDa protein, as well as histone H1, phosphatase inhibitor 1, and casein. The highly purified preparations described here should help to identify the mechanism of action of maturation-promoting factor and to elucidate the role of protein kinases in the induction of metaphase.

Purification of MPF: The Birth of Cyclin Dependent Kinases

This is *cdc2*$^+$!!
(Cdc28 in
S. cerevisiae)

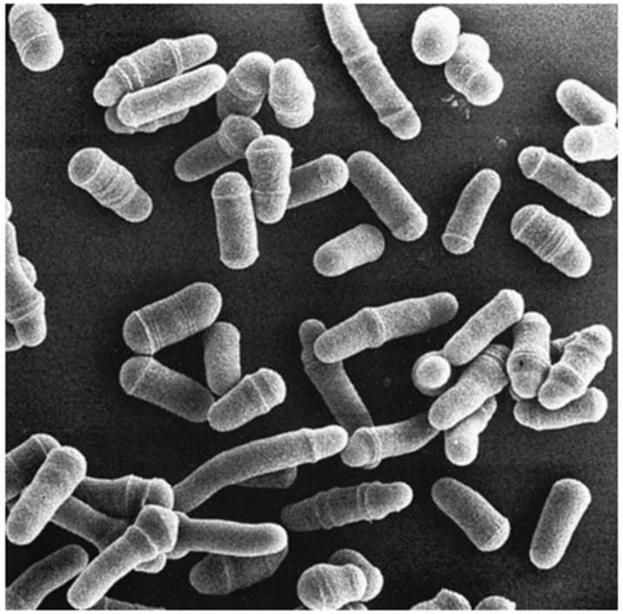


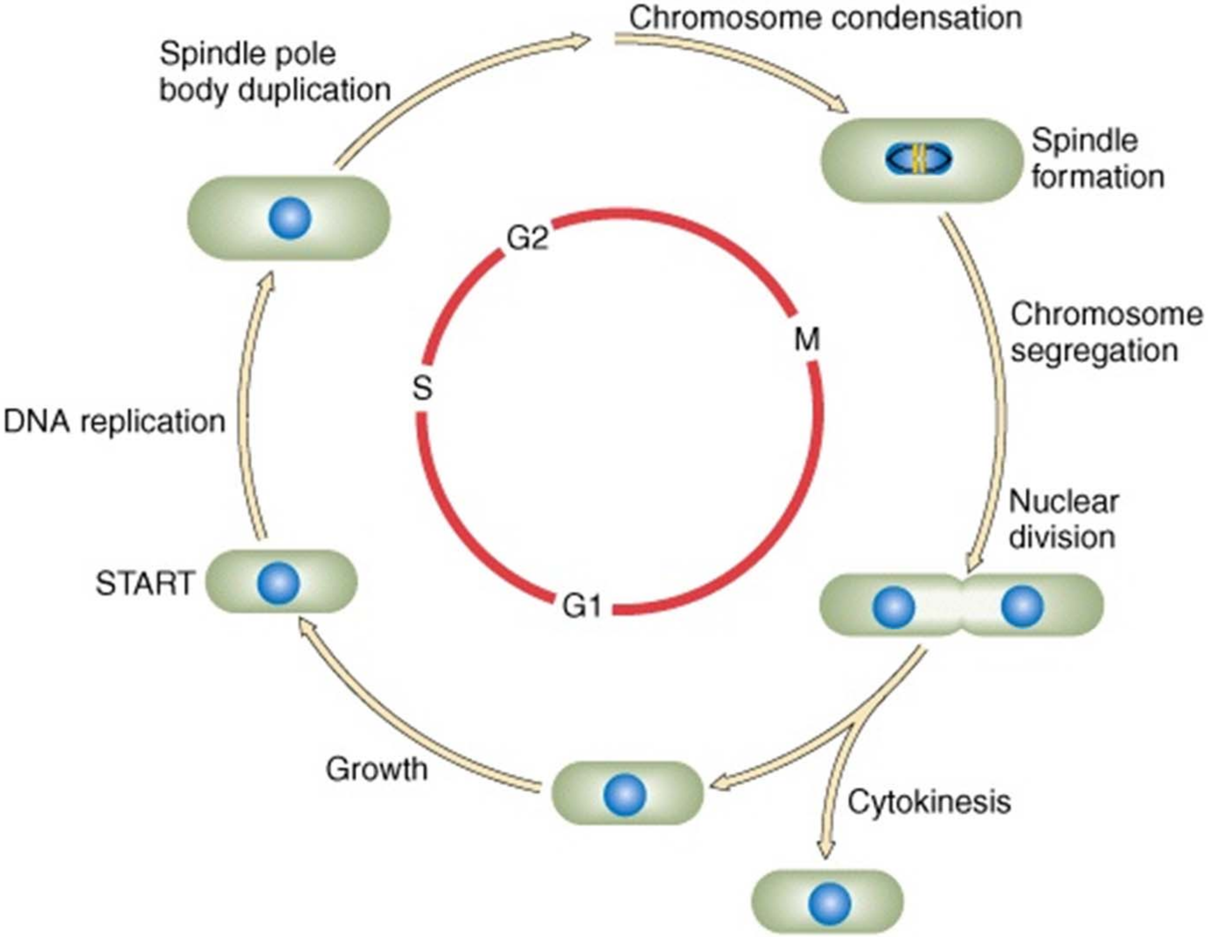
This is cyclin!!

Which = *cdc13*$^+$
in *S. pombe*

FIG. 2. Polyacrylamide gel analysis of fractions eluting from the Mono S column. A 45-μl aliquot of fractions 5–16 was incubated with $[\gamma\text{-}^{32}\text{P}]$ATP and electrophoresed through a 10% NaDodSO_4/polyacrylamide gel. (*A*) Silver-stained polyacrylamide gel of purified MPF. The activity of the fractions in the cell-free assay (NEBD) and in the oocyte microinjection assay (GVBD) is shown below the gel. NEBD is expressed in units/50 μl. GVBD is expressed as the percentage of oocytes that underwent GVBD during a 2-hr incubation in cycloheximide (0.5 μg/ml). (*B*) Autoradiograph of the silver-stained gel shown in *A*. (*C*) H1 kinase activity of purified MPF. Mono S fractions 6–15 were assayed for H1 kinase activity. The autoradiograph of the region of the gel with histone 1 is shown. Fraction 12 had a specific activity of ≈270 $\text{nmol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$.

Fission yeast: *Schizosaccharomyces pombe*





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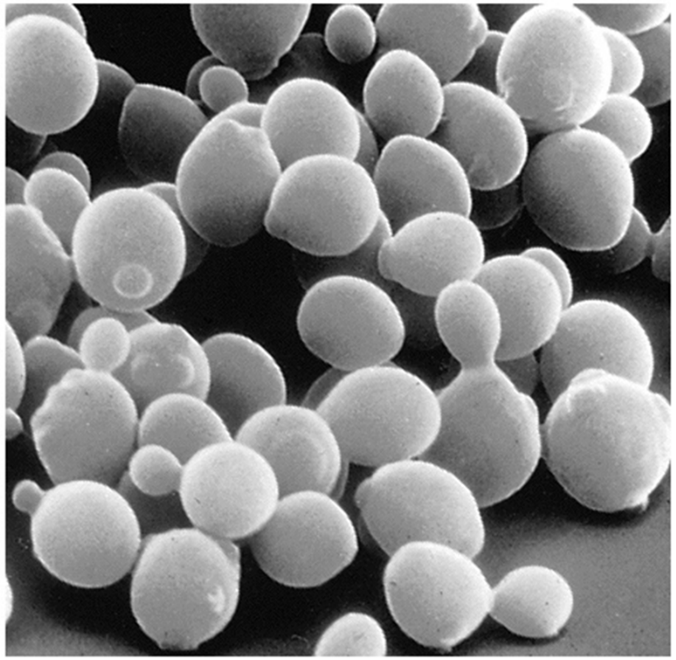
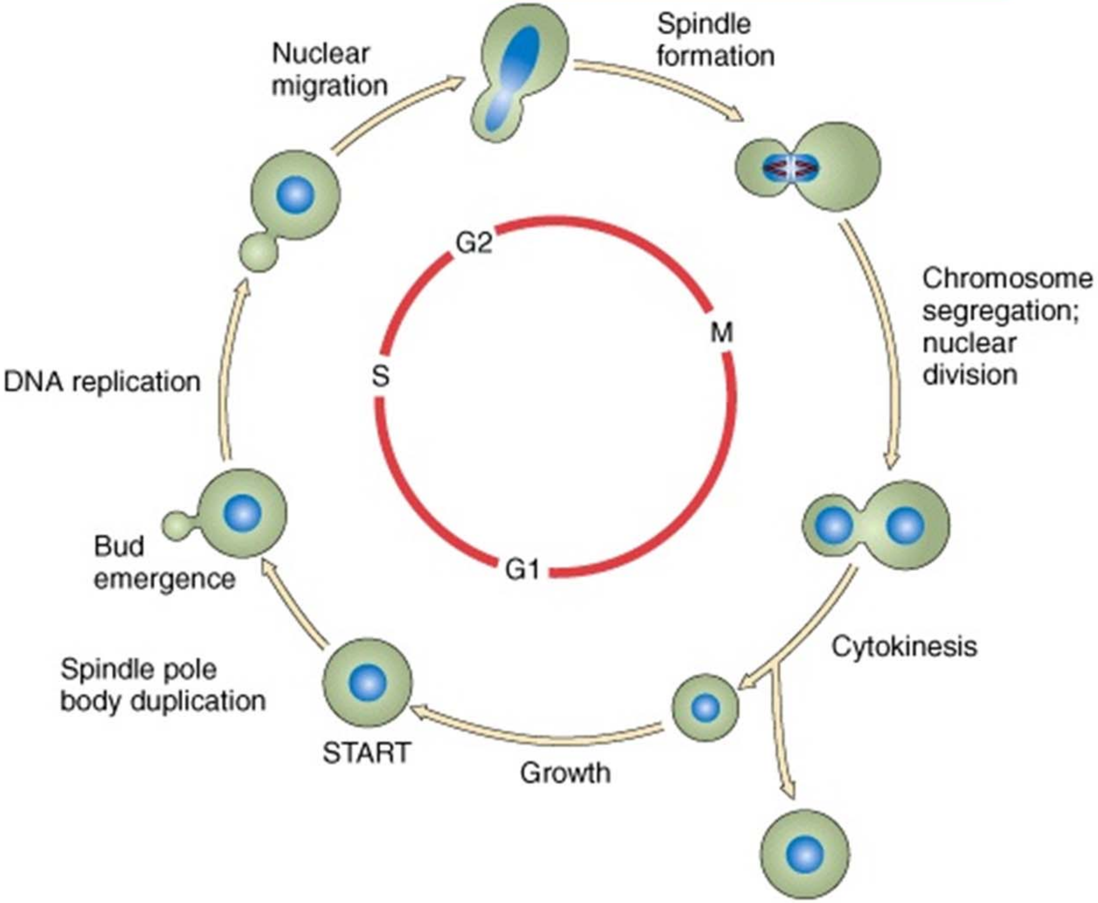
Budding Yeast *Saccharomyces cerevisiae*





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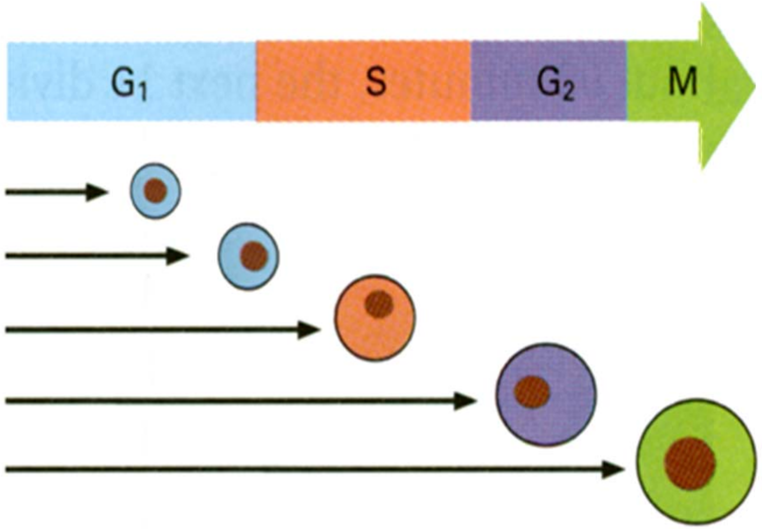
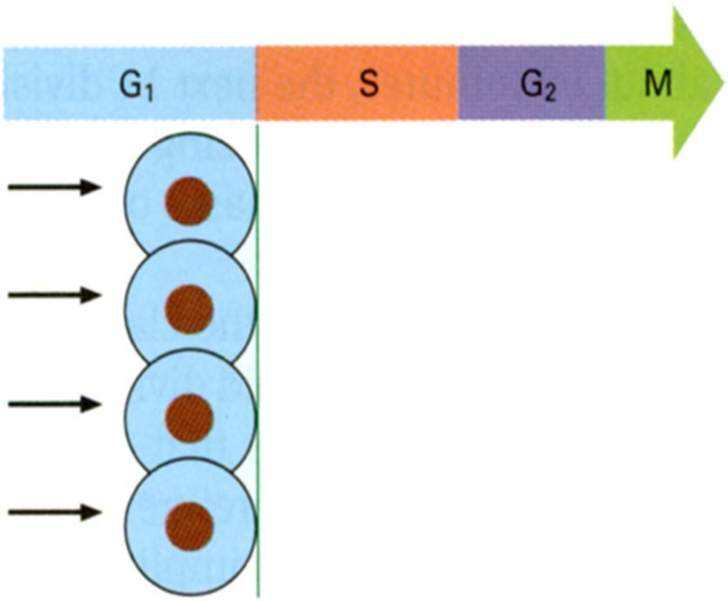
Cdc Mutants Arrest at the Same Cell Cycle Phase

Permissive (low) temperature



Restrictive (high) temperature



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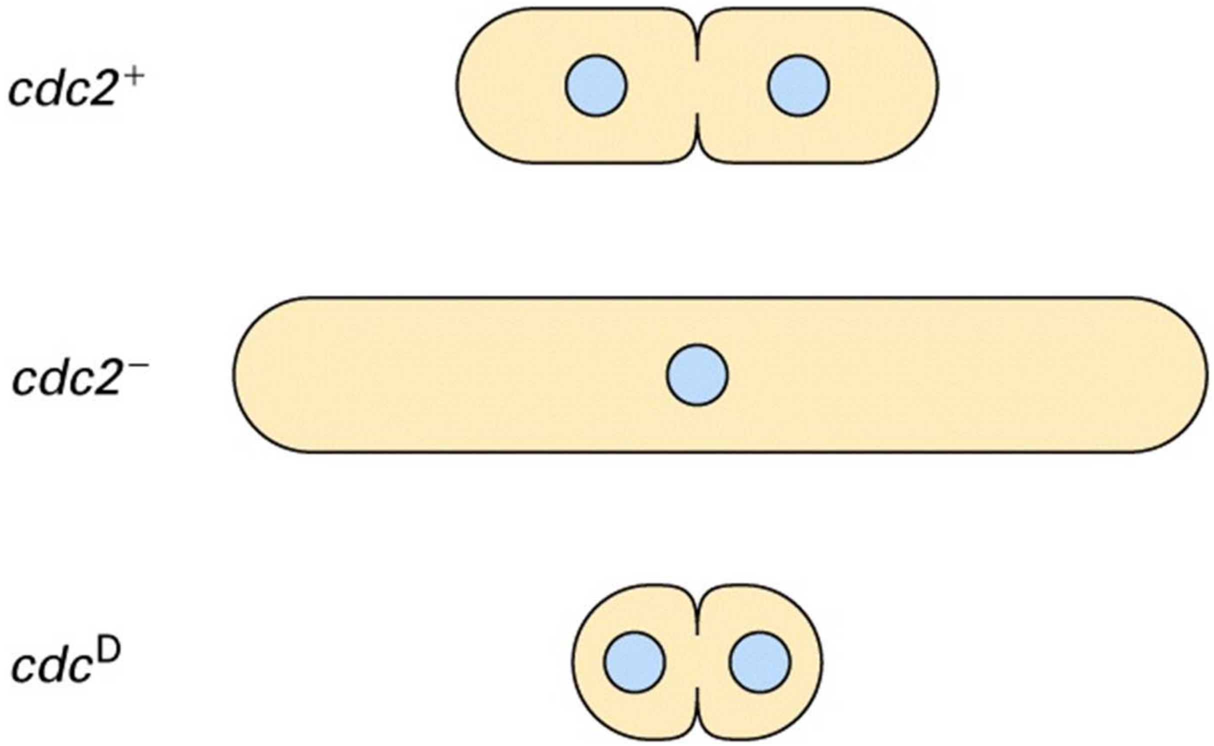
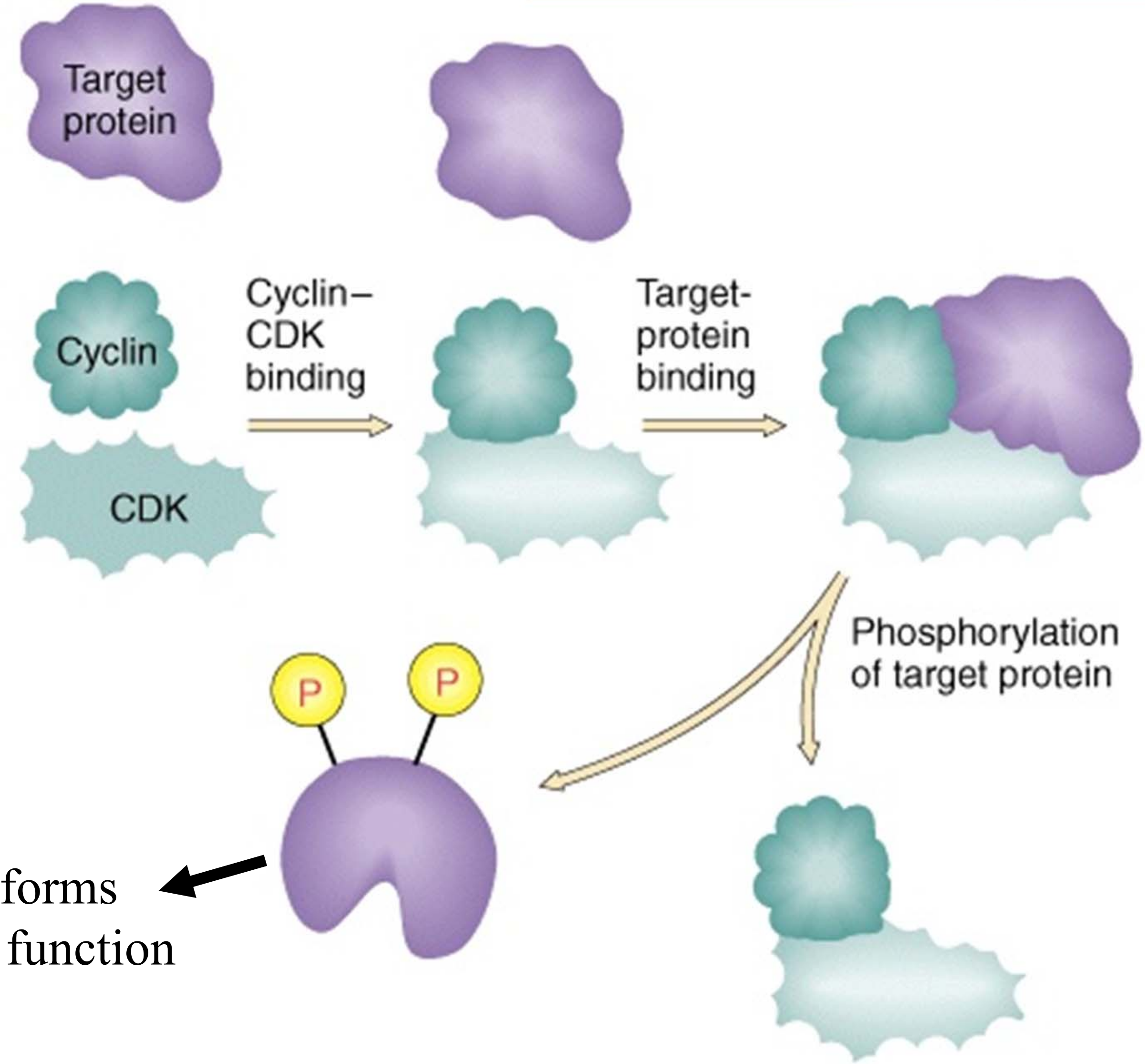
Cdc Genes Encode Proteins Needed for the G2-M Transition: Studies in *S. pombe*



cdc2$^+$ encodes a kinase

Moreover = *cdc28* in *S. cerevisiae*!

Phosphorylation of CDK Targets Changes Their Activity



Now performs
a cell cycle function

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Jak jsou CDK regulovány?

1. prostřednictvím syntézy a odbourávání cyklinů
2. fosforylací
3. pomocí CDK inhibitory proteins (CKIs)

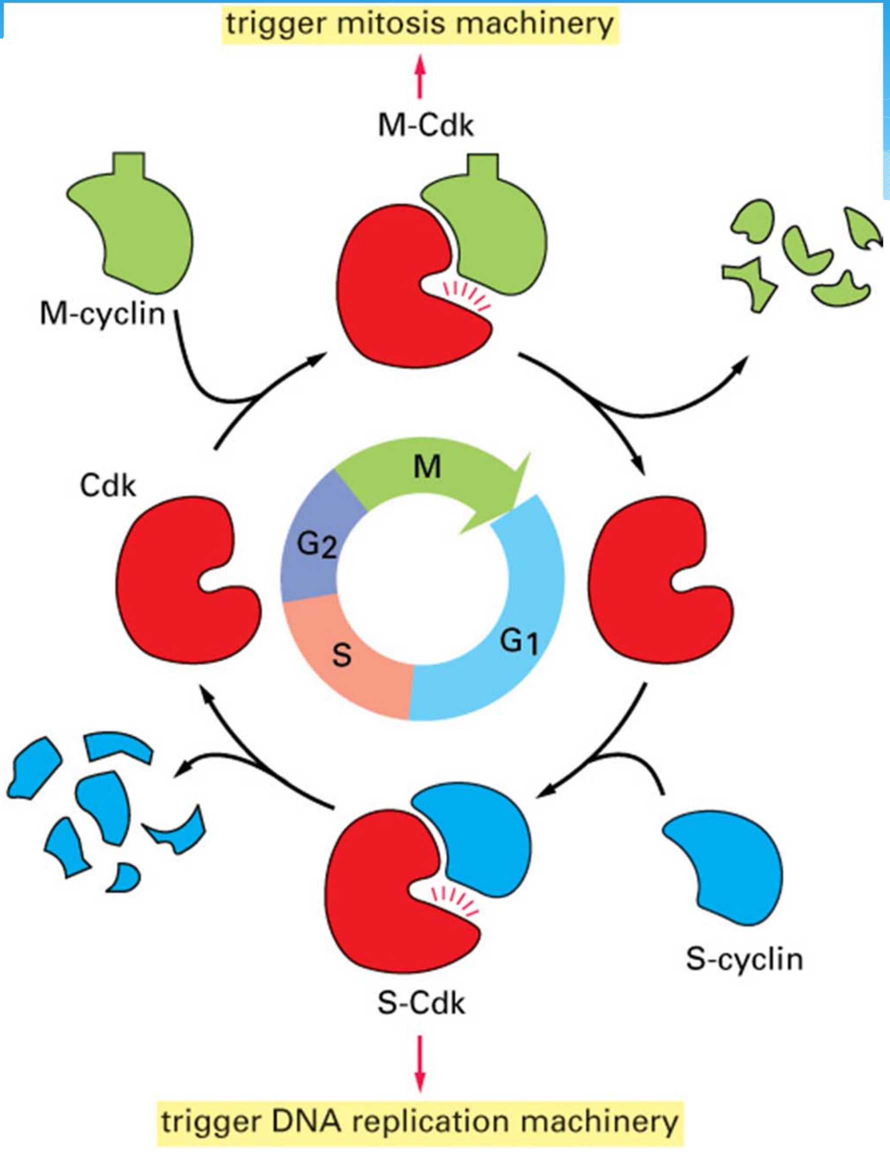
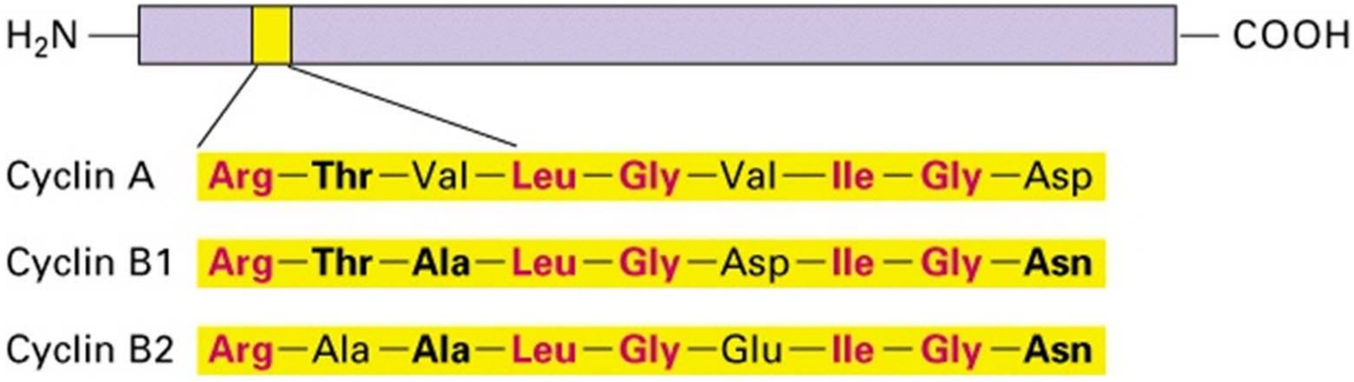


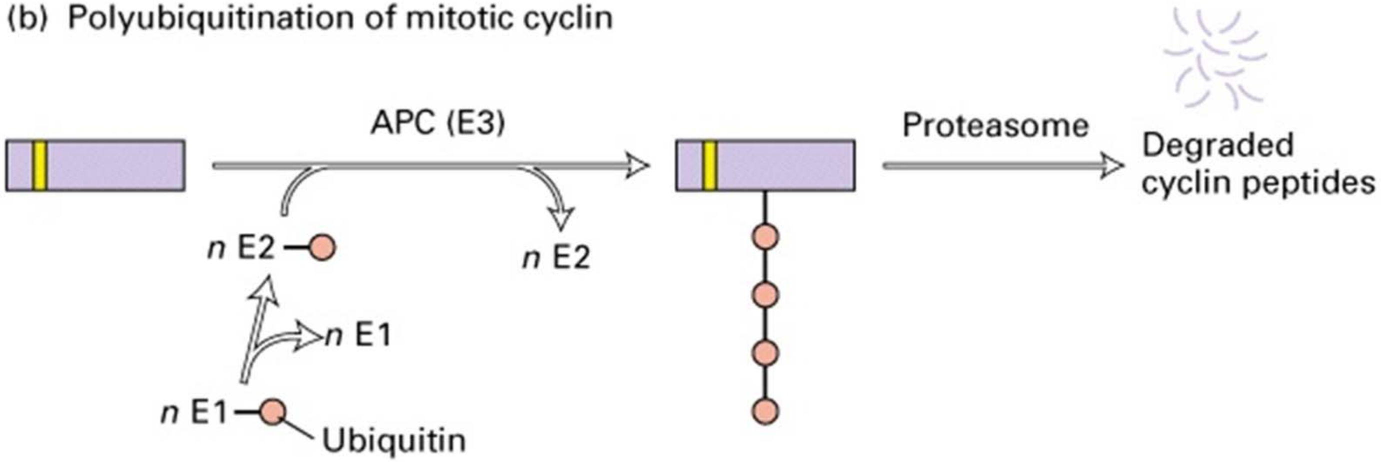
Figure 17–16. Molecular Biology of the Cell, 4th Edition.

Cyclin Destruction is Controlled by Ubiquitination

(a) Mitotic cyclin destruction box



(b) Polyubiquitination of mitotic cyclin



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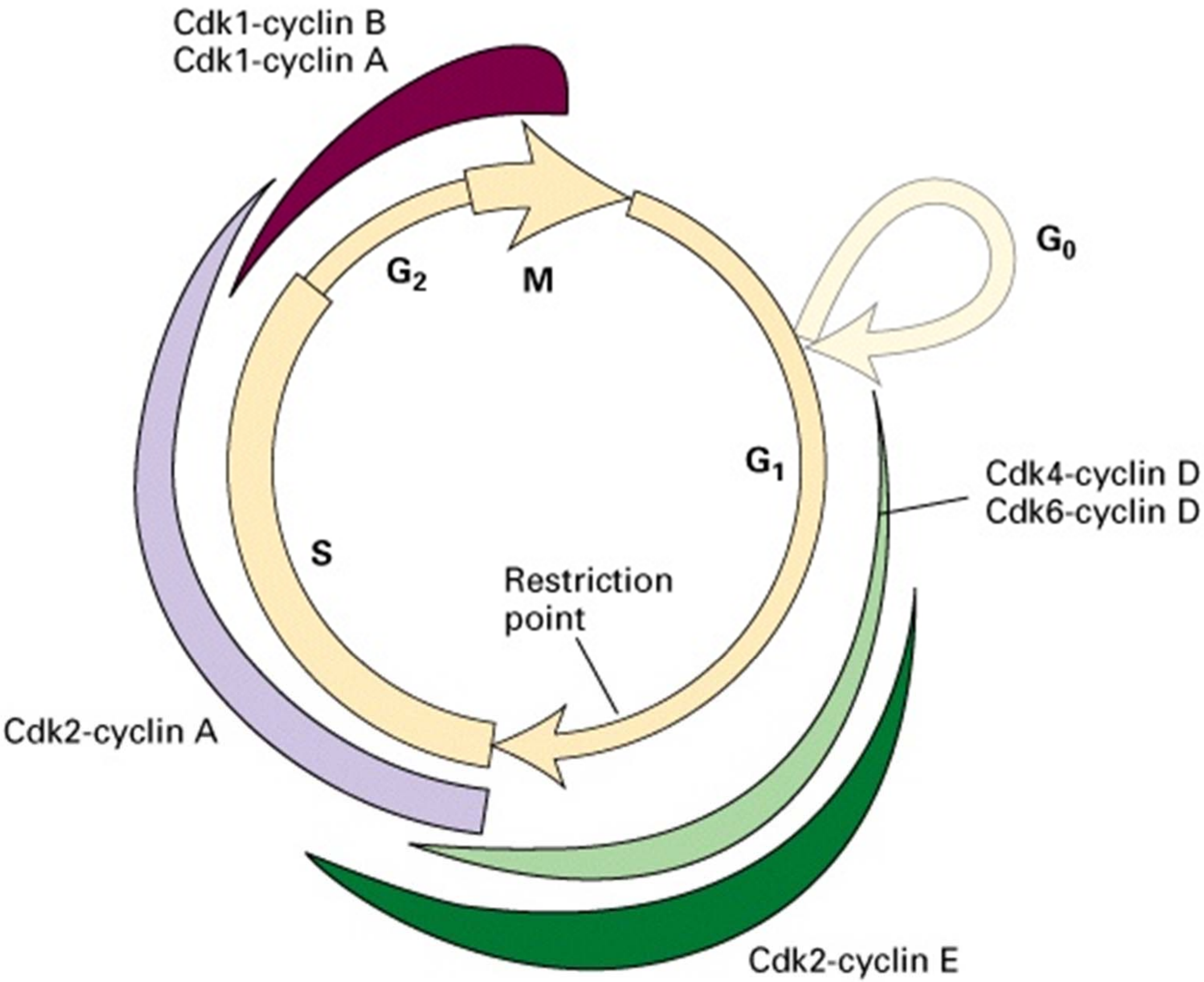
Představují cykliny jediný způsob regulace CDK?

Exprese cyklinů v jednotlivých fázích BC



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CDK jsou regulovány fosforylací

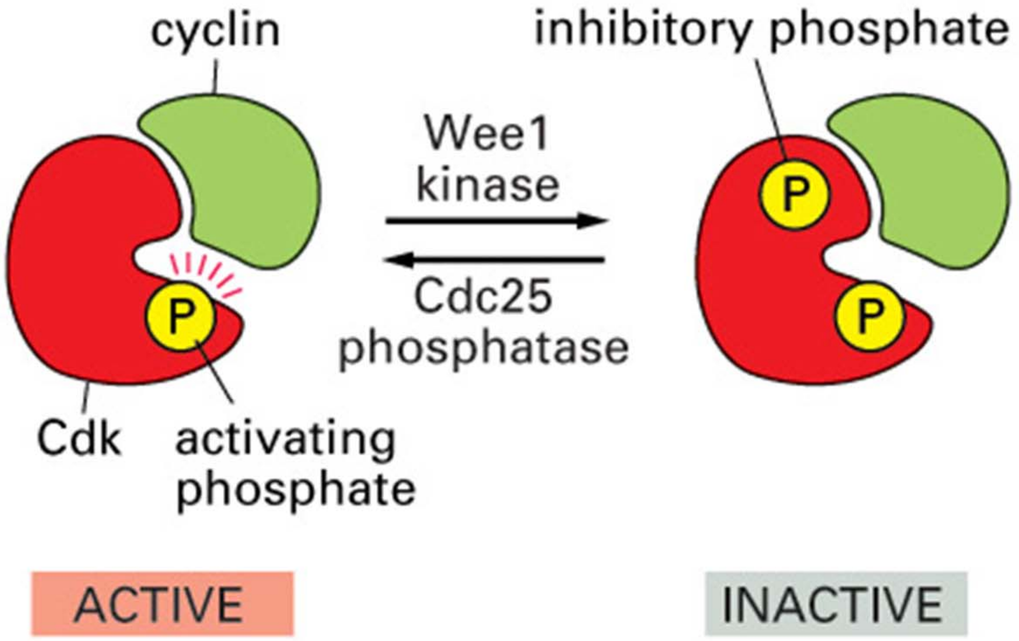
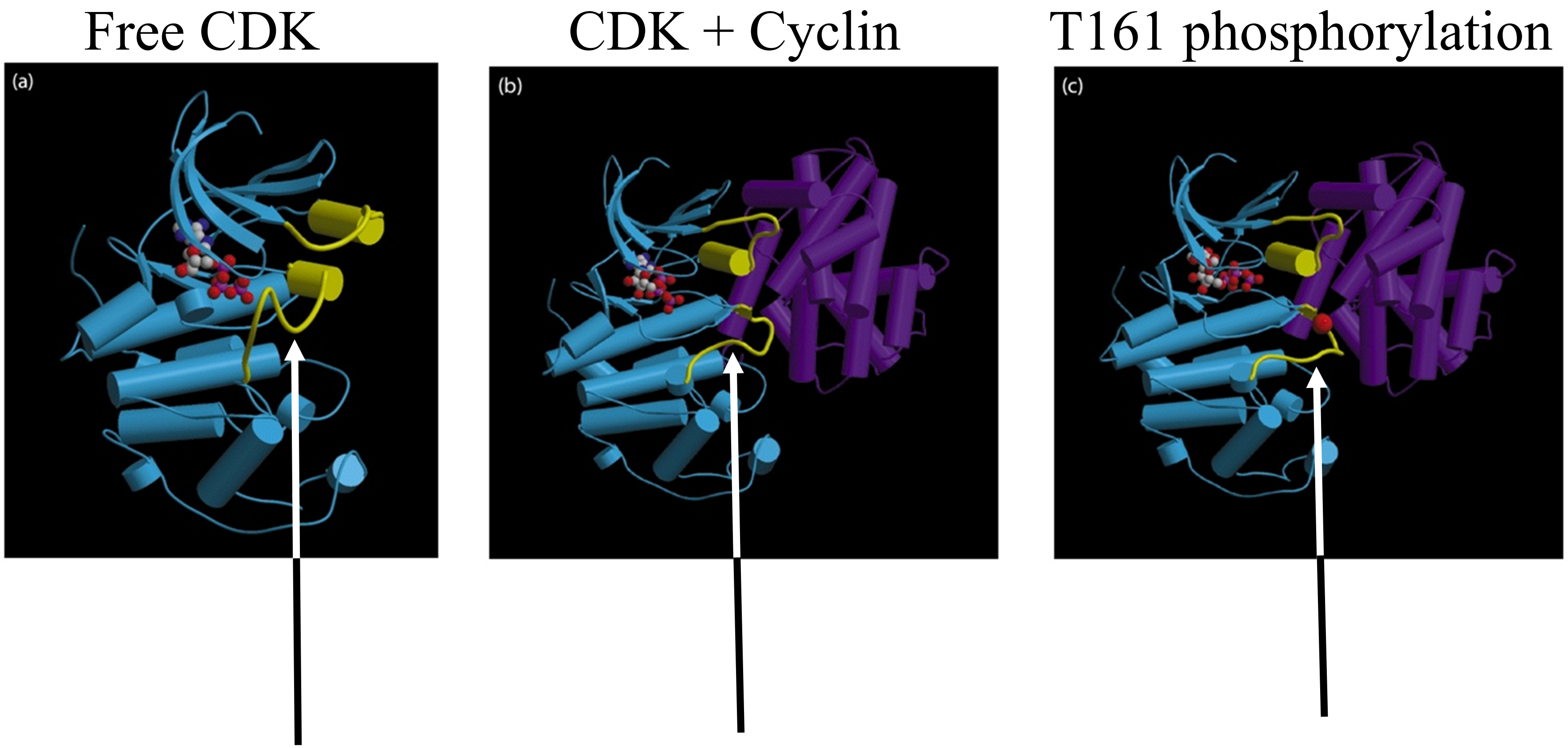


Figure 17–18. Molecular Biology of the Cell, 4th Edition.

Conformational Changes Associated with CDK Phosphorylation



Free CDK

CDK + Cyclin

T161 phosphorylation

The T-loop blocks substrate access

Binding of cyclin moves the T-loop

Phosporylation moves the T-loop more

Cyclin Dependent Kinase Inhibitors (CKIs)

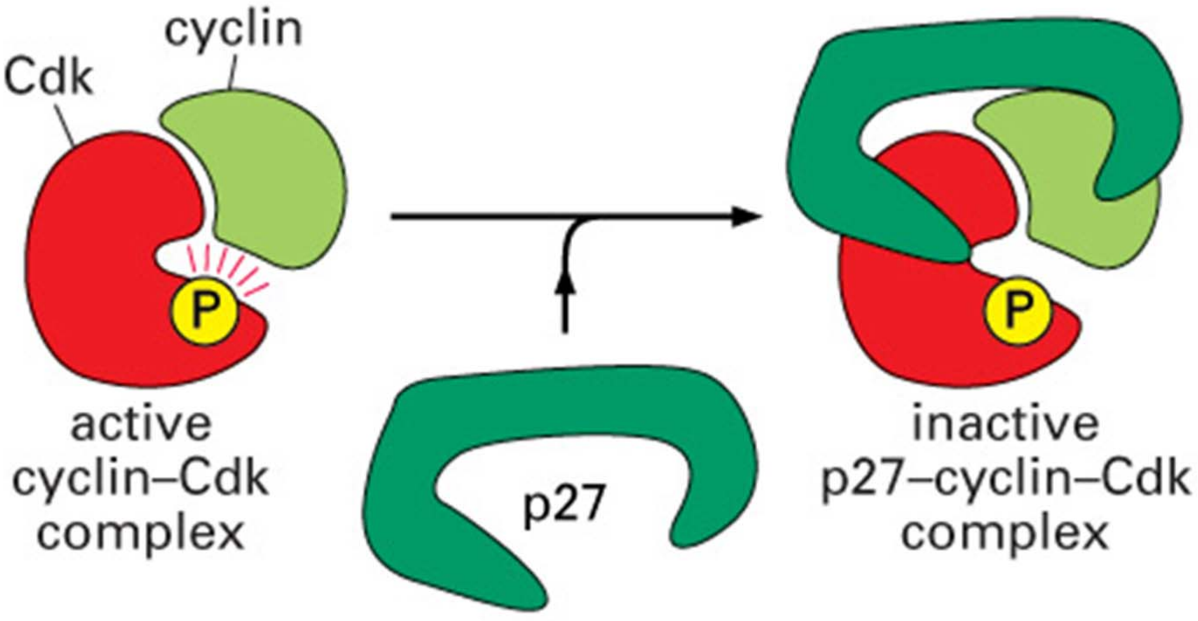
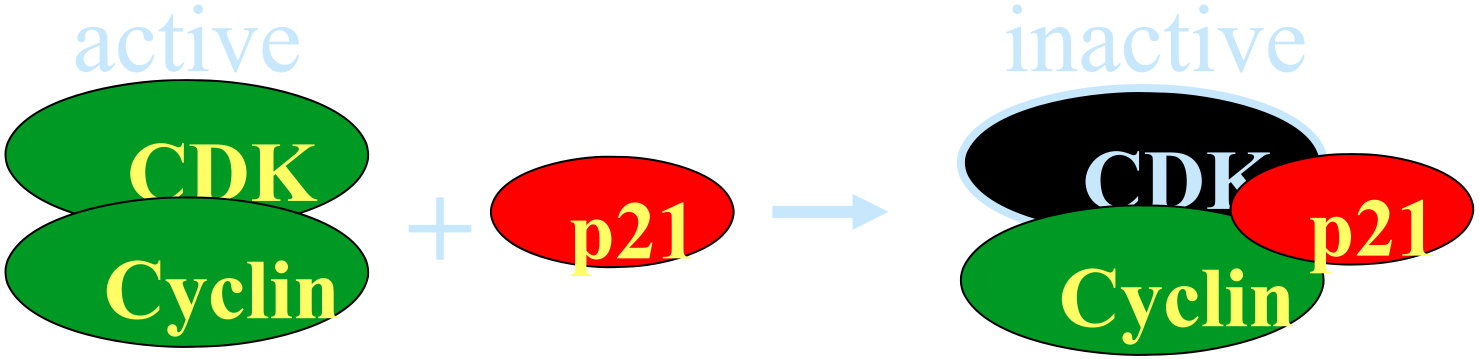
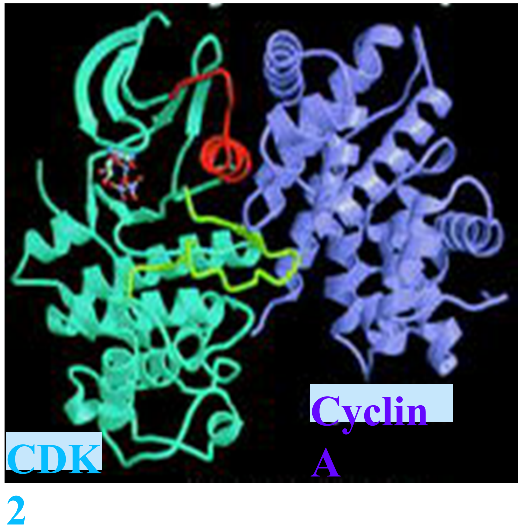


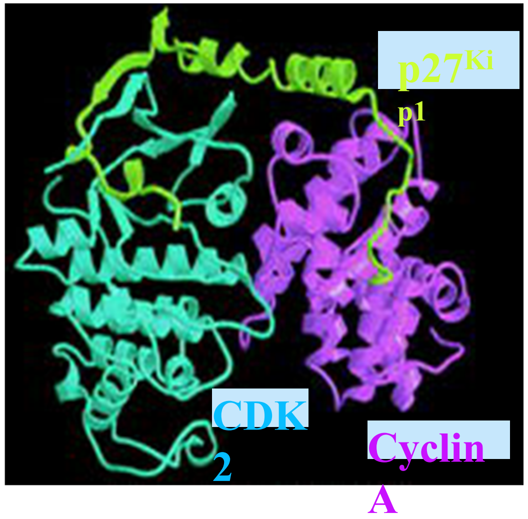
Figure 17–19. Molecular Biology of the Cell, 4th Edition.

The p21 Family of CDK inhibitors ($p21^{CIP1/WAF1}$, $p27^{KIP1}$, $p57^{KIP2}$)



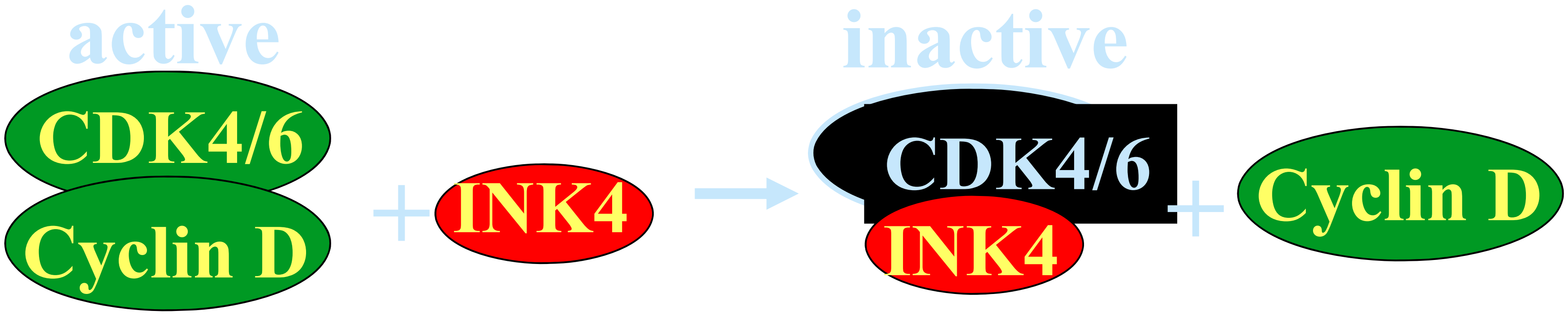


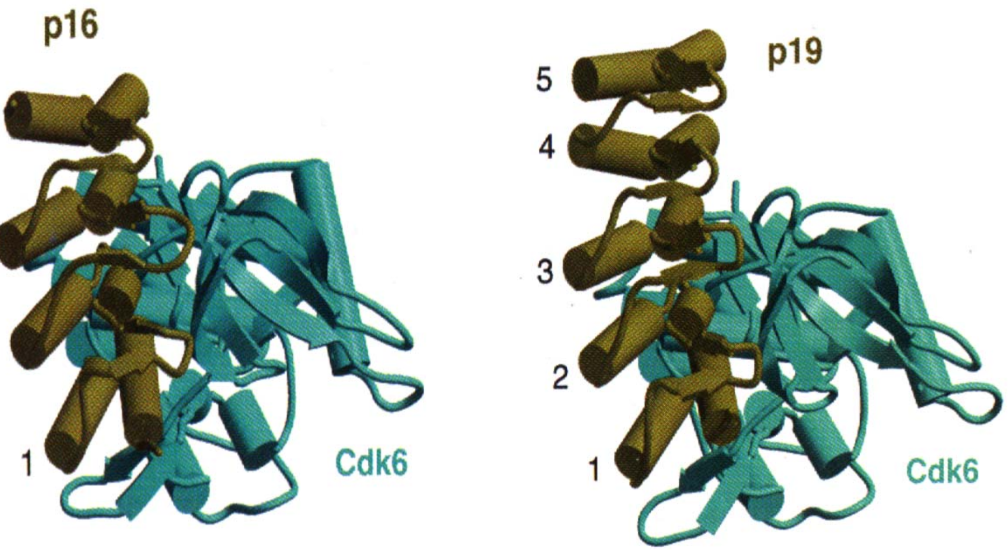
Jeffrey et al. (1995) *Nature* 376:313



Russo et al. (1996) *Nature* 382:325

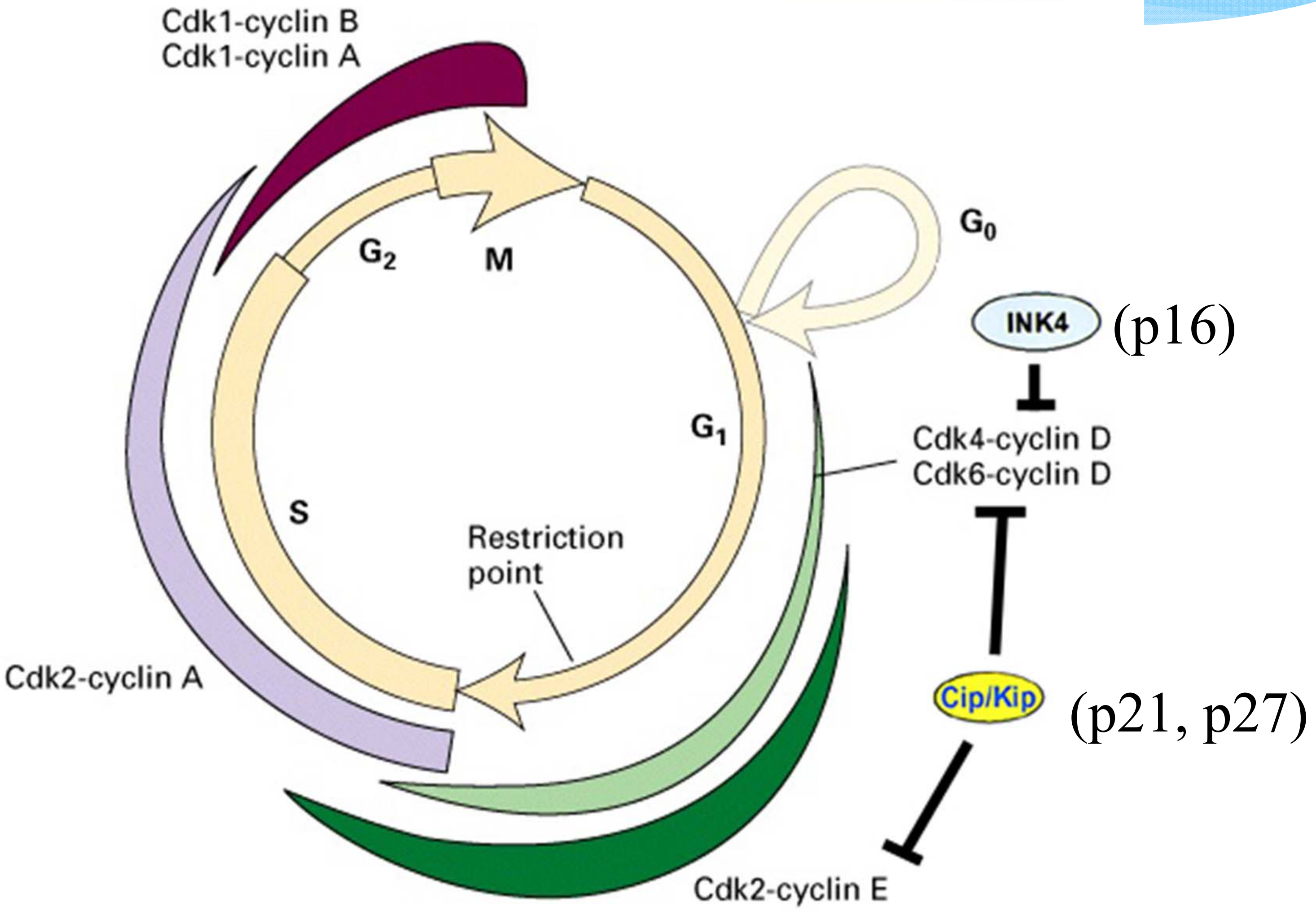
The INK4 Family of CDK inhibitors (p16$^{\text{INK4a}}$, p15$^{\text{INK4b}}$, p18$^{\text{INK4c}}$, p19$^{\text{INK4d}}$)





Russo et al. (1998) *Nature* 395:237
Brotherton et al. (1998) *Nature* 395:244

CKIs Regulate the G1-S Transition



(p16)

(p21, p27)

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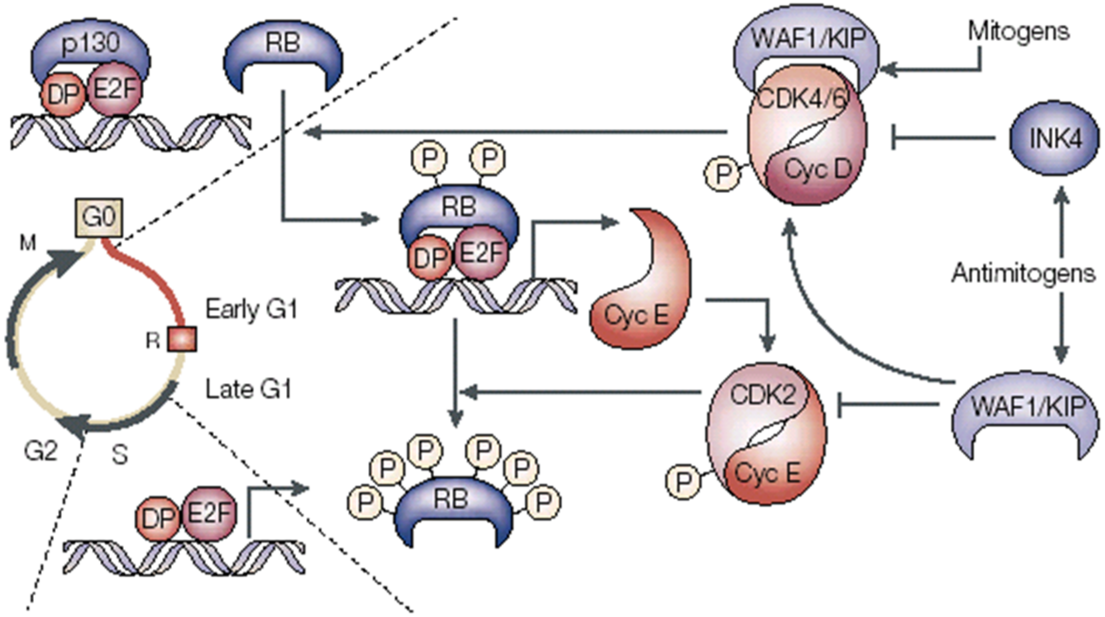
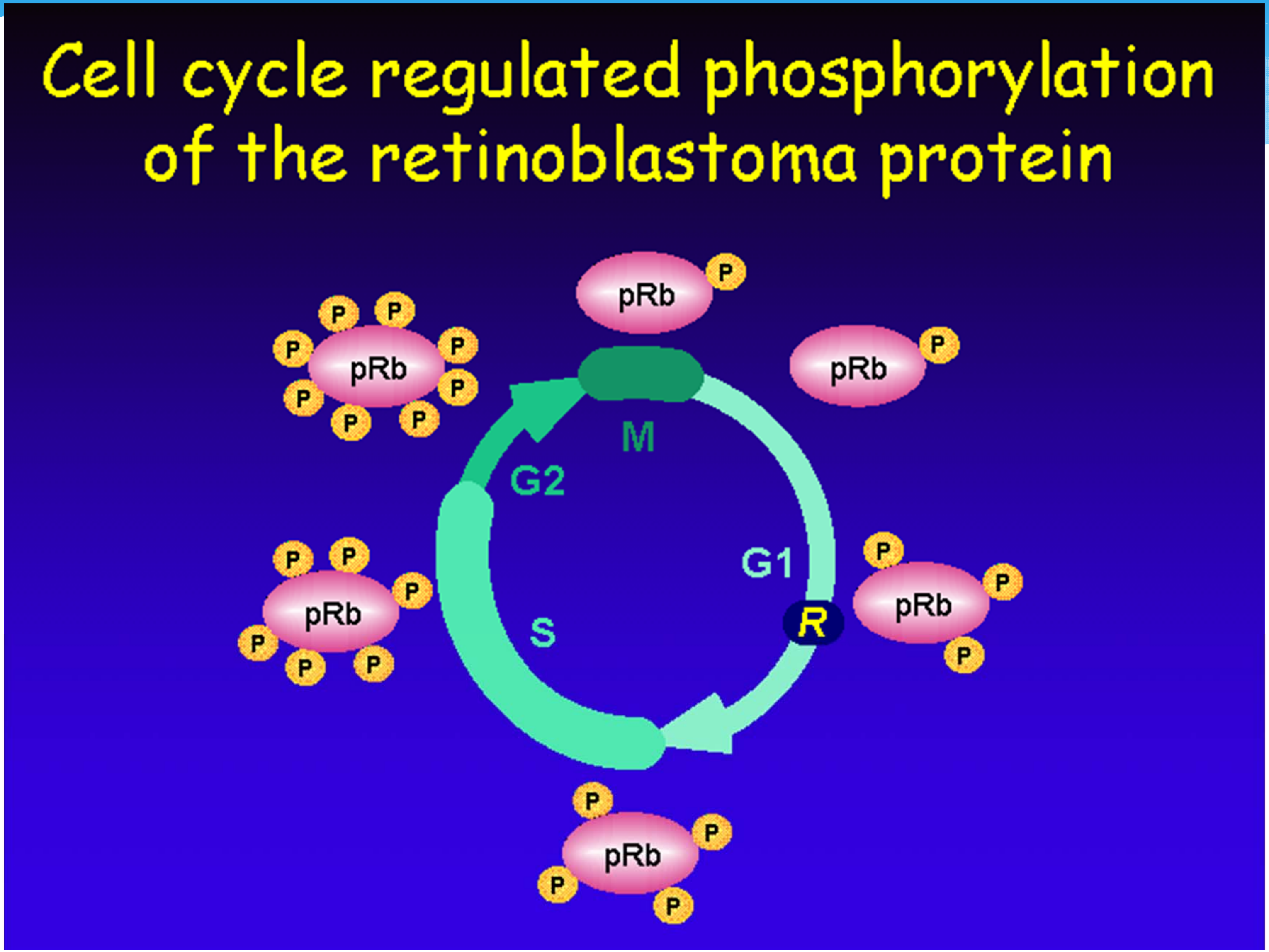


Figure 1 | **Regulation of G1 and the G1/S transition.** In quiescent, G0 cells, E2F–DP transcription factors are bound to p130, the principal pocket protein in these cells, which keeps them inactive. In G1, however, RB–E2F–DP complexes predominate. Mitogenic signalling results in cyclin D (Cyc) synthesis, formation of active CDK4/6–cyclin-D complexes and initial phosphorylation of RB. Partially phosphorylated RB still binds to E2F–DP, but the transcription factor is still able to transcribe some genes, such as cyclin E, presumably due to impaired repression. Cyclin E binds to and activates CDK2. It is generally accepted that CDK2-dependent phosphorylation of RB results in its complete inactivation, which allows induction of the E2F-responsive genes that are needed to drive cells through the G1/S transition and to initiate DNA replication. INK4 and WAF1/KIP proteins can inhibit CDK4/6 or CDK2 kinases, respectively, following specific antimitogenic signals. The CDK4/6 complexes can also bind WAF1/KIP inhibitors, while remaining active. This sequesters them from CDK2, which facilitates its full activation. R represents the restriction point that separates the mitogen-dependent early G1 phase from the mitogen-independent late G1 phase.

Nature Reviews Cancer 2001, 1:222



Cell cycle regulated phosphorylation of the retinoblastoma protein

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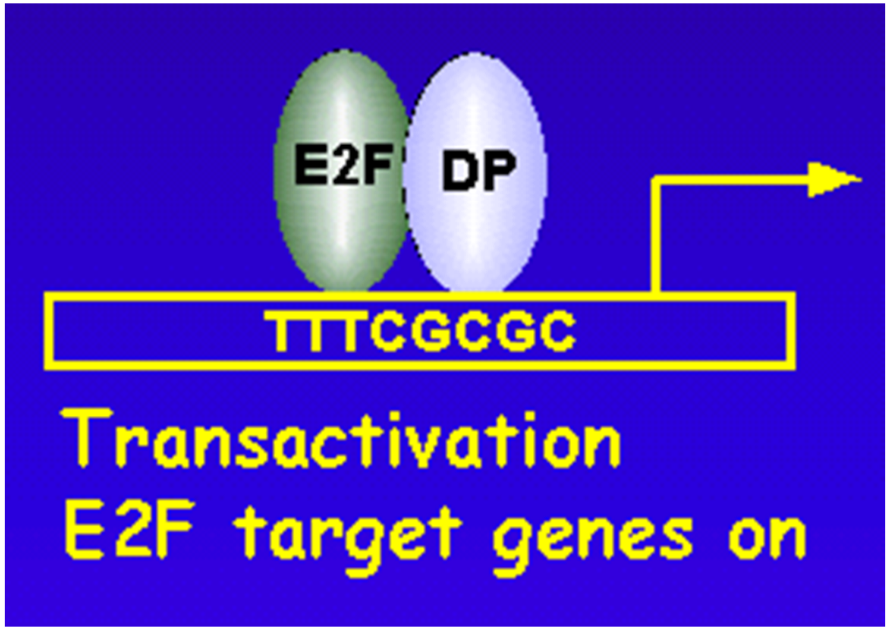
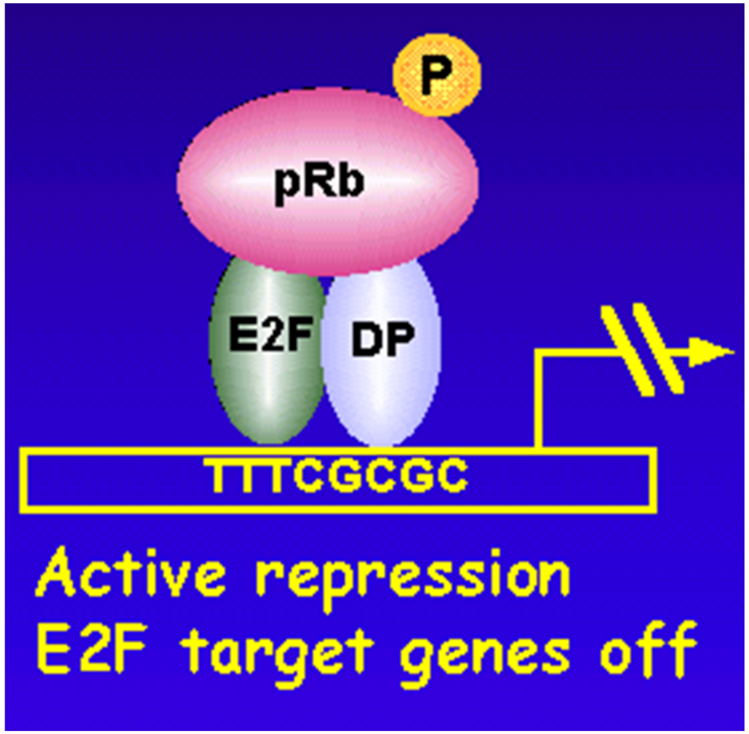
pRB Binds to the E2F Transcription Factor





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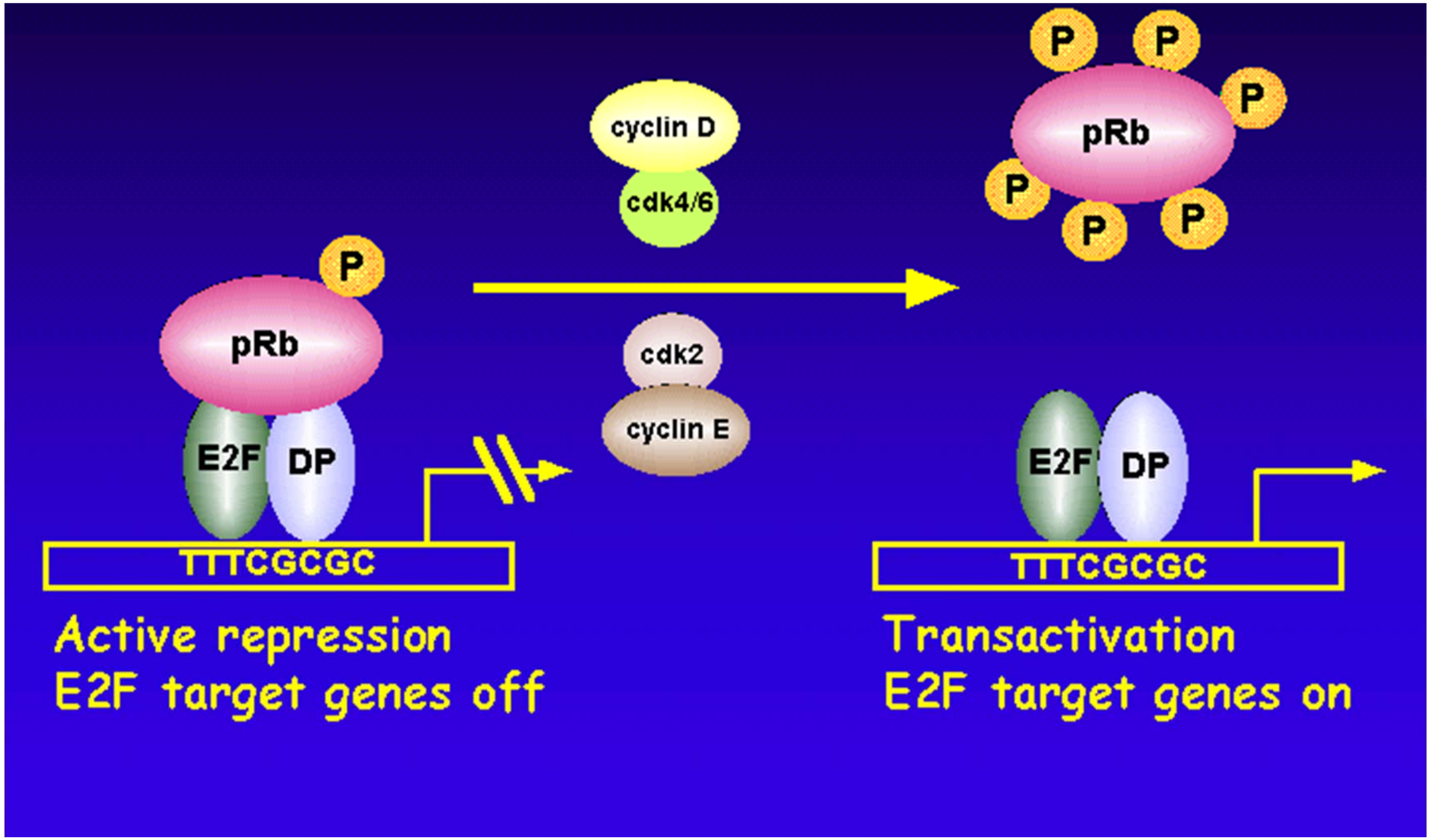
G1 Cyclin CDKs Phosphorylate pRB



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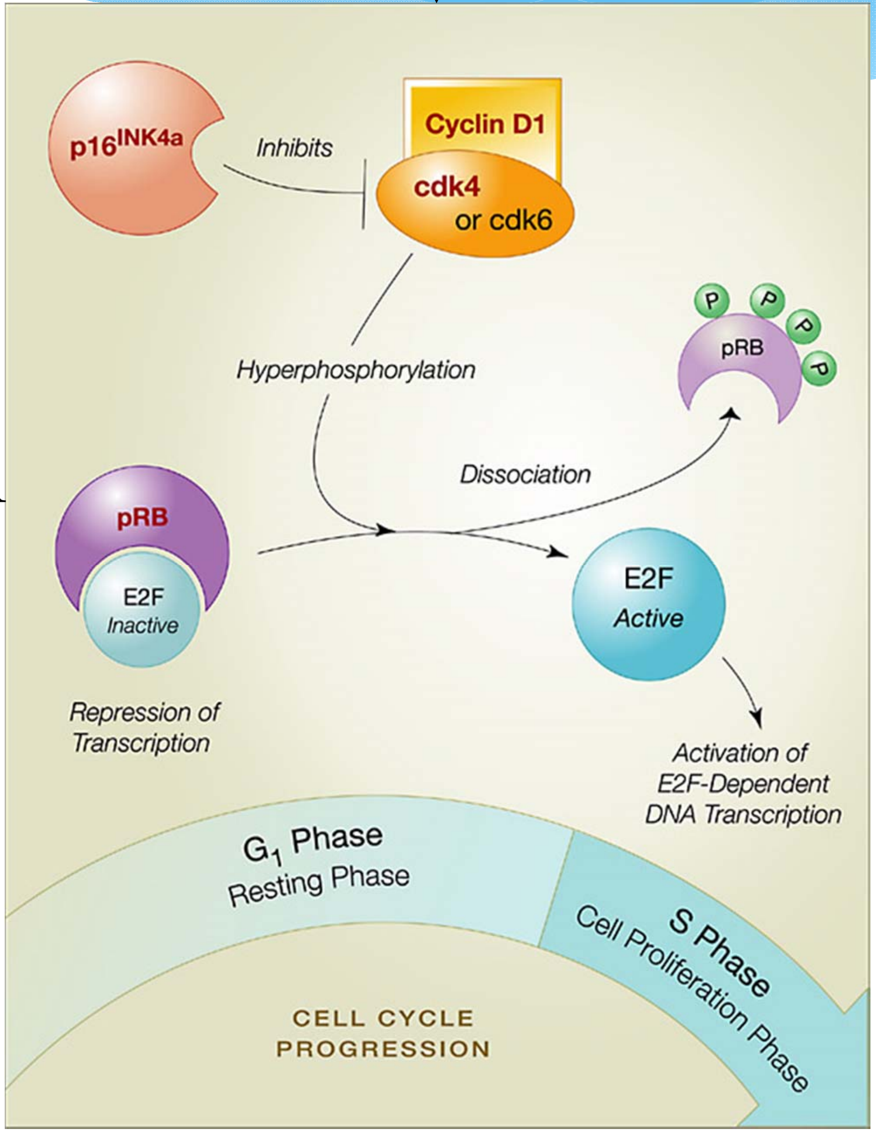
p16 Regulates pRB Phosphorylation

Proliferation Signals



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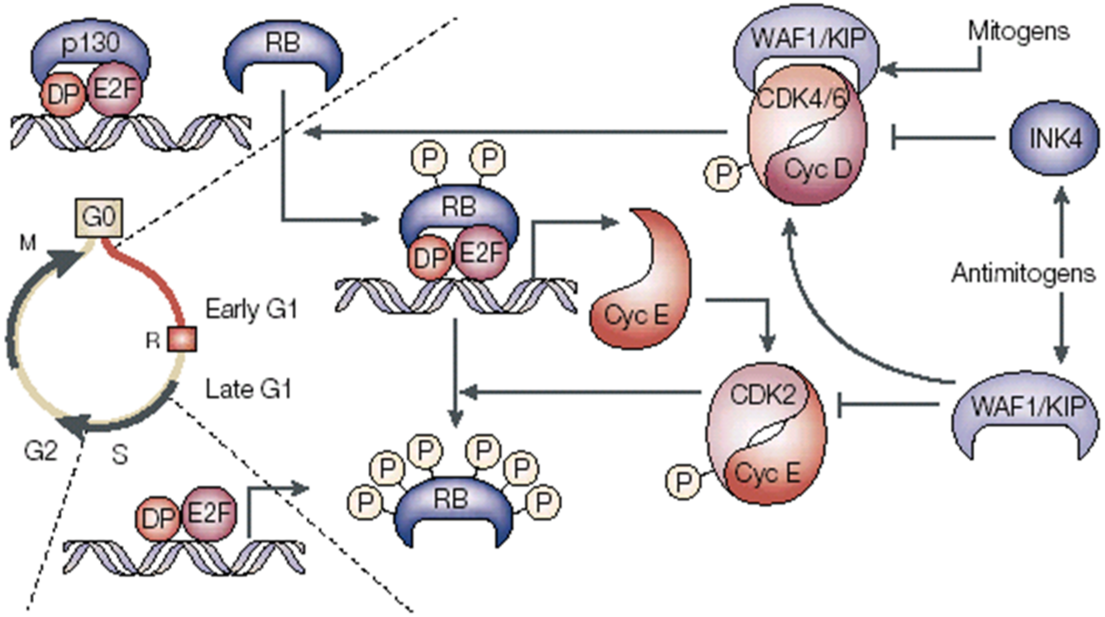


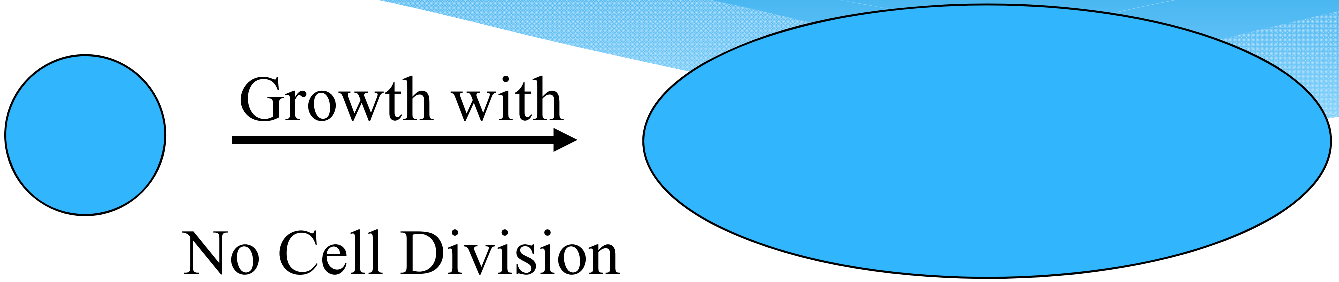
Figure 1 | **Regulation of G1 and the G1/S transition.** In quiescent, G0 cells, E2F–DP transcription factors are bound to p130, the principal pocket protein in these cells, which keeps them inactive. In G1, however, RB–E2F–DP complexes predominate. Mitogenic signalling results in cyclin D (Cyc) synthesis, formation of active CDK4/6–cyclin-D complexes and initial phosphorylation of RB. Partially phosphorylated RB still binds to E2F–DP, but the transcription factor is still able to transcribe some genes, such as cyclin E, presumably due to impaired repression. Cyclin E binds to and activates CDK2. It is generally accepted that CDK2-dependent phosphorylation of RB results in its complete inactivation, which allows induction of the E2F-responsive genes that are needed to drive cells through the G1/S transition and to initiate DNA replication. INK4 and WAF1/KIP proteins can inhibit CDK4/6 or CDK2 kinases, respectively, following specific antimitogenic signals. The CDK4/6 complexes can also bind WAF1/KIP inhibitors, while remaining active. This sequesters them from CDK2, which facilitates its full activation. R represents the restriction point that separates the mitogen-dependent early G1 phase from the mitogen-independent late G1 phase.

Nature Reviews Cancer 2001, 1:222

Kontrola buněčného cyklu úzce souvisí s:

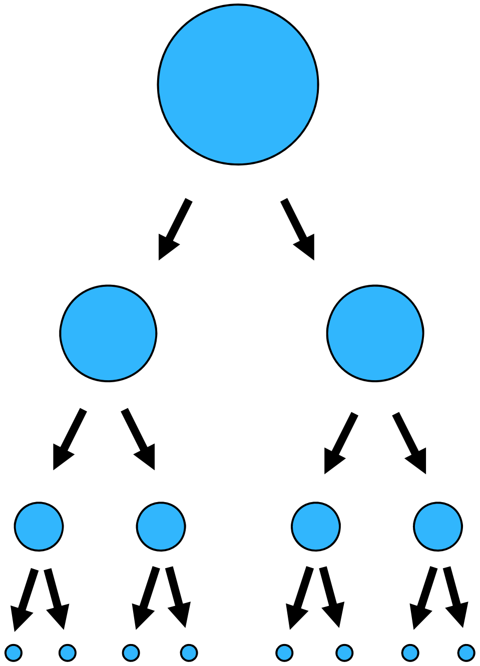
- kontrolou buněčného růstu;
- přítomností růstových faktorů a dalších růstových stimulů a živin;
- působením ostatních buněk populace a mezibuněčné hmoty.

The Difference Between Growth and Cell Division



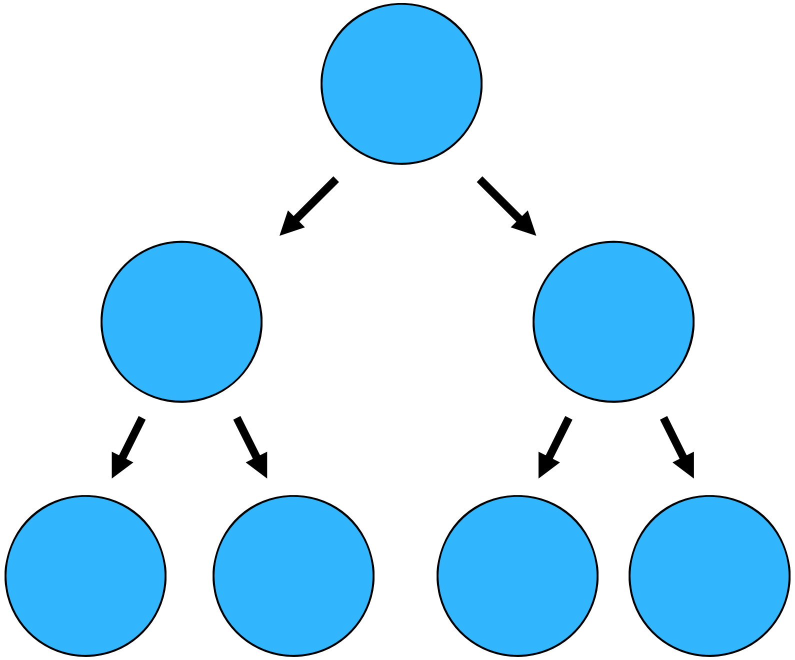
Growth with

No Cell Division



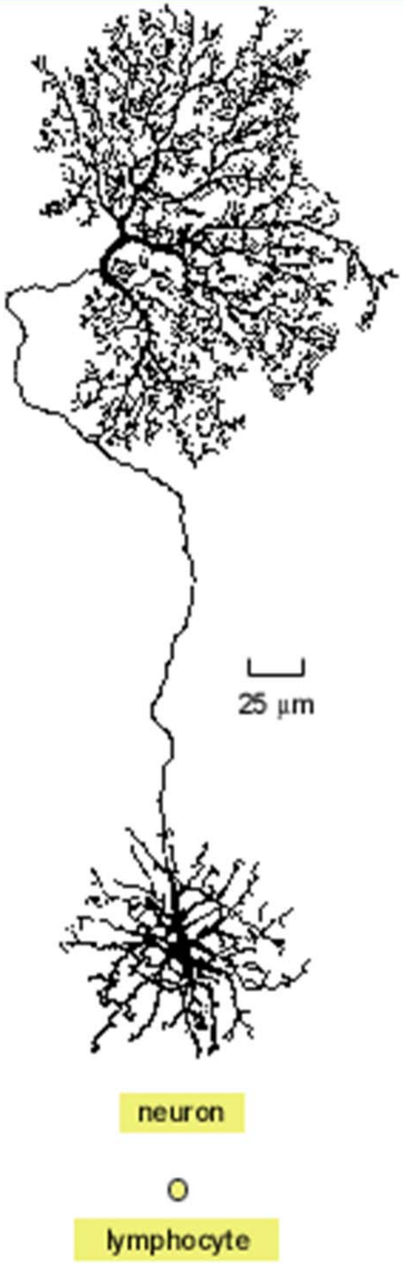
Cell Division

No Growth



Cell Division + Growth =

Proliferation!



Growth with No Cell Division: A Differentiated Neuron

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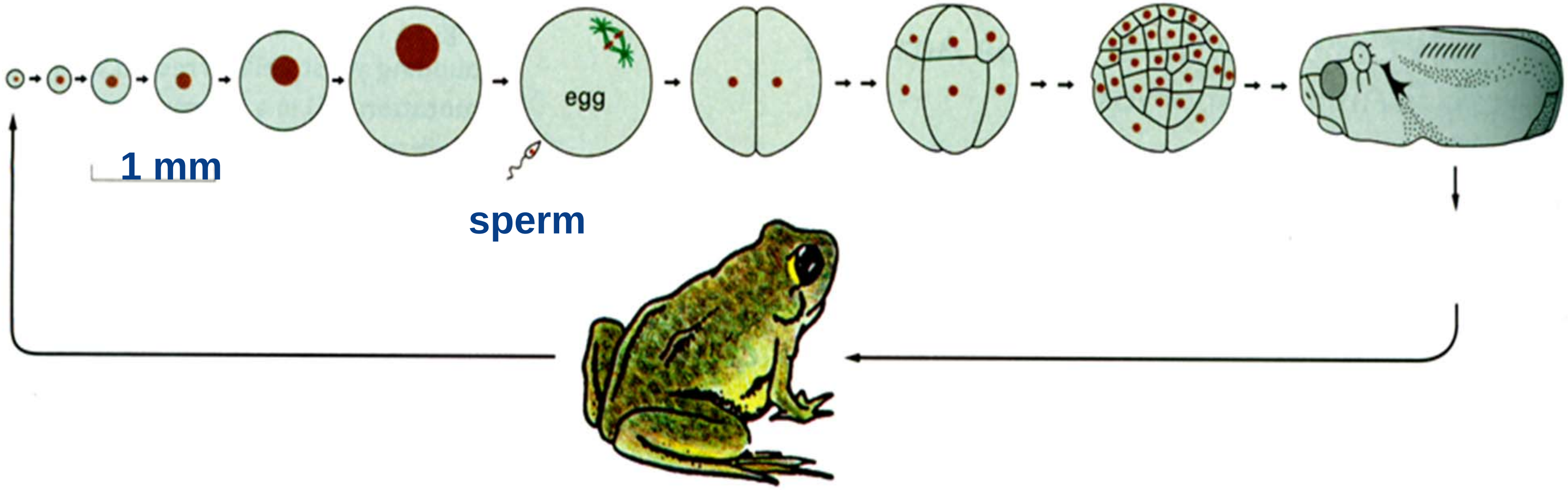
Cell Division with No Growth:
Early Development



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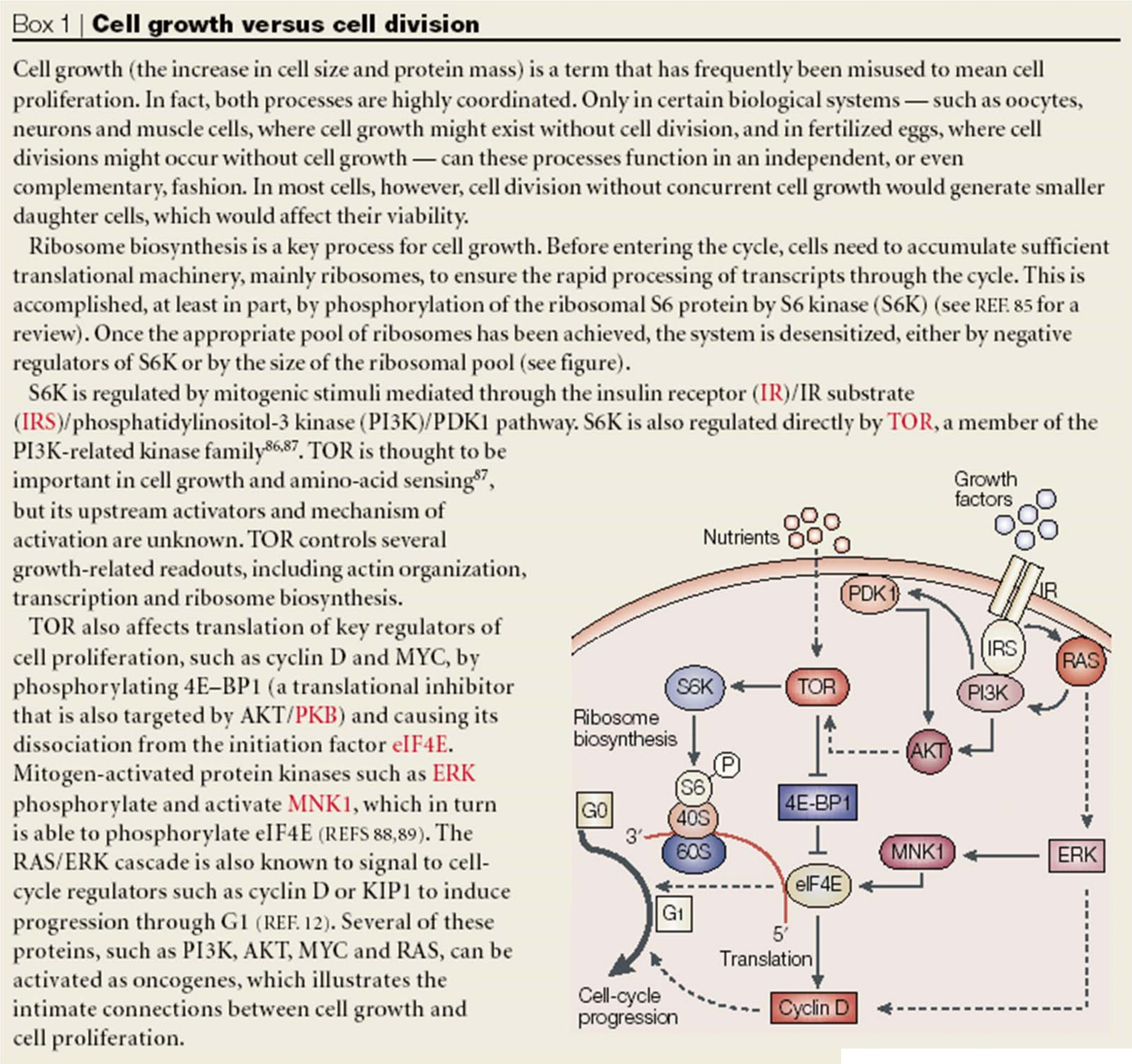


Box 1 | **Cell growth versus cell division**

Cell growth (the increase in cell size and protein mass) is a term that has frequently been misused to mean cell proliferation. In fact, both processes are highly coordinated. Only in certain biological systems — such as oocytes, neurons and muscle cells, where cell growth might exist without cell division, and in fertilized eggs, where cell divisions might occur without cell growth — can these processes function in an independent, or even complementary, fashion. In most cells, however, cell division without concurrent cell growth would generate smaller daughter cells, which would affect their viability.

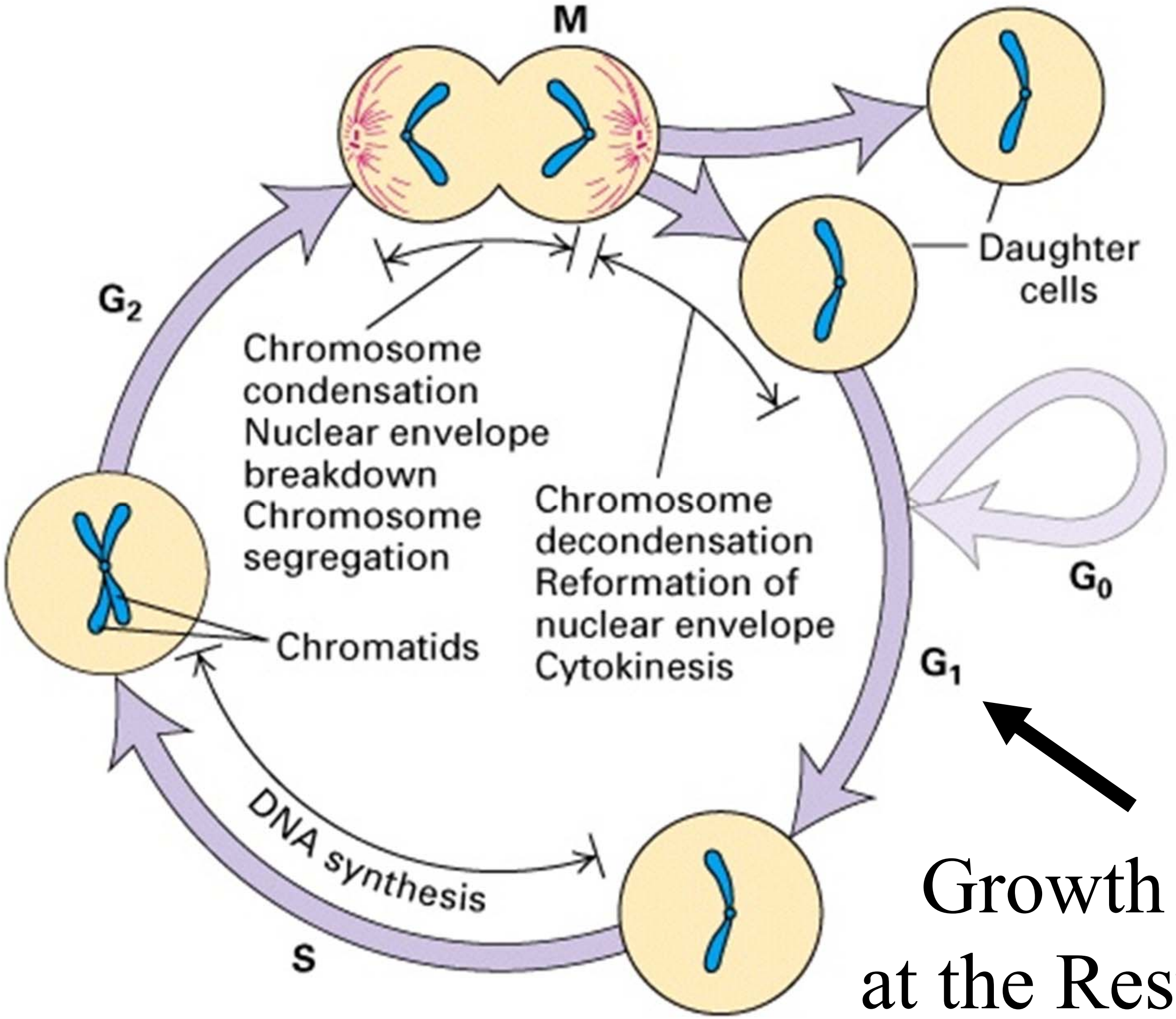
Ribosome biosynthesis is a key process for cell growth. Before entering the cycle, cells need to accumulate sufficient translational machinery, mainly ribosomes, to ensure the rapid processing of transcripts through the cycle. This is accomplished, at least in part, by phosphorylation of the ribosomal S6 protein by S6 kinase (S6K) (see REF. 85 for a review). Once the appropriate pool of ribosomes has been achieved, the system is desensitized, either by negative regulators of S6K or by the size of the ribosomal pool (see figure).

S6K is regulated by mitogenic stimuli mediated through the insulin receptor (IR)/IR substrate (IRS)/phosphatidylinositol-3 kinase (PI3K)/PDK1 pathway. S6K is also regulated directly by TOR, a member of the PI3K-related kinase family[86,87]. TOR is thought to be important in cell growth and amino-acid sensing[87], but its upstream activators and mechanism of activation are unknown. TOR controls several growth-related readouts, including actin organization, transcription and ribosome biosynthesis.

TOR also affects translation of key regulators of cell proliferation, such as cyclin D and MYC, by phosphorylating 4E–BP1 (a translational inhibitor that is also targeted by AKT/PKB) and causing its dissociation from the initiation factor eIF4E. Mitogen-activated protein kinases such as ERK phosphorylate and activate MNK1, which in turn is able to phosphorylate eIF4E (REFS 88,89). The RAS/ERK cascade is also known to signal to cell-cycle regulators such as cyclin D or KIP1 to induce progression through G1 (REF. 12). Several of these proteins, such as PI3K, AKT, MYC and RAS, can be activated as oncogenes, which illustrates the intimate connections between cell growth and cell proliferation.

Nature Reviews Cancer 2001, 1:222

Growth Factors Induce Cell Cycle Progression



Growth Factors act
at the Restriction Point

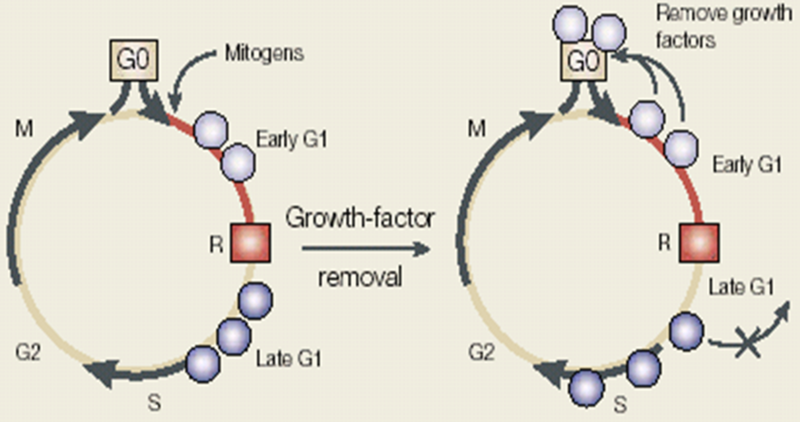
Dr. B. Duronio, The University of North Carolina at Chapel Hill

Box 2 | **The Restriction Point**

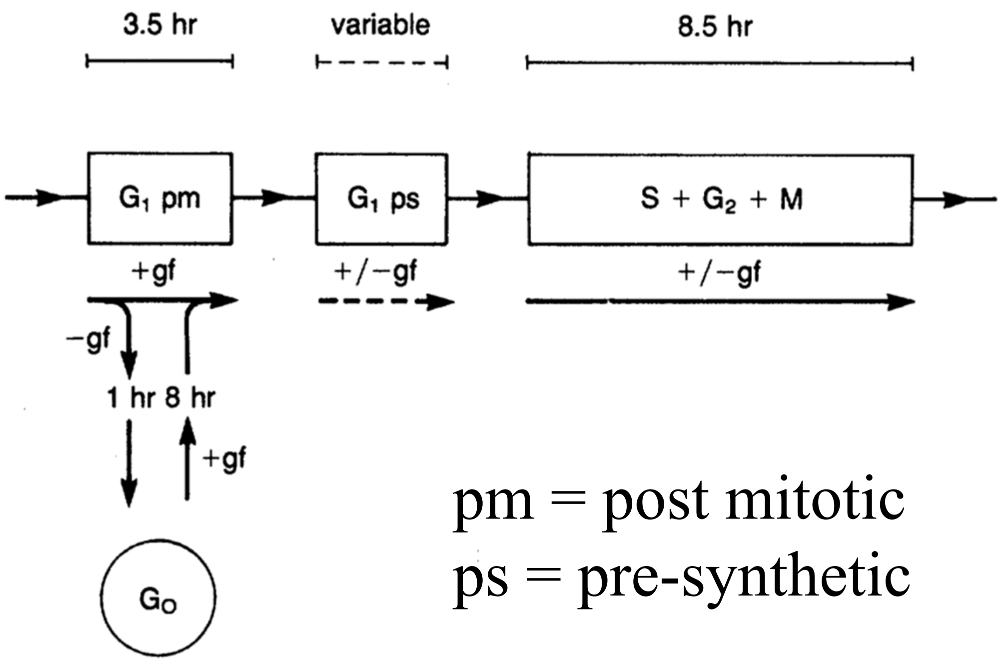
The term 'Restriction Point' was coined in 1974 by Arthur Pardee[90] to define a specific event in G1 after which cultured cells could proliferate independently of mitogenic stimuli. Briefly, cultured mammalian cells that had undergone mitosis within the previous 3 hours could be prevented from progressing through the cell cycle by growth-factor starvation or moderate inhibition of protein synthesis. These cells then re-entered the cell cycle after re-stimulation with growth factors. However, if the cells had undergone cell division more than 4 hours before, they did not respond to mitogen deprivation and advanced through the cell cycle with the same kinetics as unstarved cells. It was postulated that the latter cells had 'passed' the restriction point (R). Today, R is often used to divide the early and late G1 phases.

R does not represent a checkpoint as originally defined in yeast[2,91]. In culture cells, R occurs 3–4 hours after mitosis (see figure). However, entry into S phase is usually initiated 5–13 hours after mitosis. This variability is characteristic of the late G1 phase and accounts for most of the observed differences in the length of the cell cycle. Indeed, the differential kinetics of these two transitions indicates distinct control mechanisms. The molecular events that allow cells to pass R have not been well defined. However, members of the RB family are likely to be important, as ablation of this gene family eliminates R[92,93].

It has been postulated that loss of regulation of R is critical in cancer. R normally prevents cells from entering the cycle until they have accumulated a certain threshold of mitogen-induced events, so loosening of R control due to mutations in G1 regulators or other, as yet unidentified, genes would allow cells to enter the cycle even in the absence of adequate mitogenic signalling, leading to unscheduled proliferation. Validation of this model will require definition of the molecular players that regulate R.



Nature Reviews Cancer 2001, 1:222

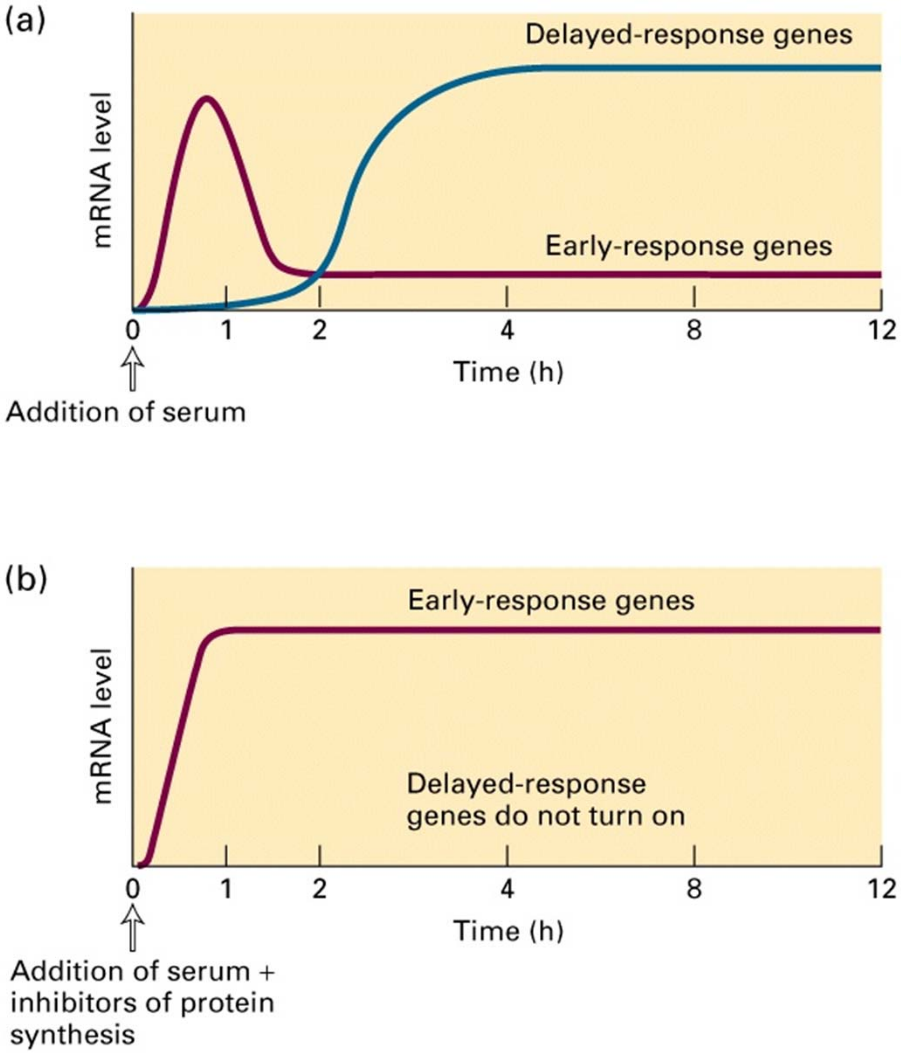


pm = post mitotic
ps = pre-synthetic

FIG. 7. Schematic model of cell cycle in Swiss 3T3 cells. During the first 3.5-hr after mitosis (G_1pm), the cell makes the decision whether or not to progress through the cell cycle. This decision depends on the presence of growth factors (gf). If the cell senses a lack of growth factors (−gf) in G_1pm, it will leave the cell cycle within 15–60 min and enter a state of quiescence (G_0) from which it takes 8 hr to reenter the cycle after the growth factor level in the environment again becomes optimal (+gf) for proliferation. Once the cell has entered G_1ps, it will eventually initiate DNA synthesis. However, G_1ps is highly variable in length and in fact responsible for most of the variability in the duration of G_1 and of the whole cell cycle.

Zetterberg and Larsson, PNAS 82:5365 (1985)

Growth Factors Induce Gene Expression



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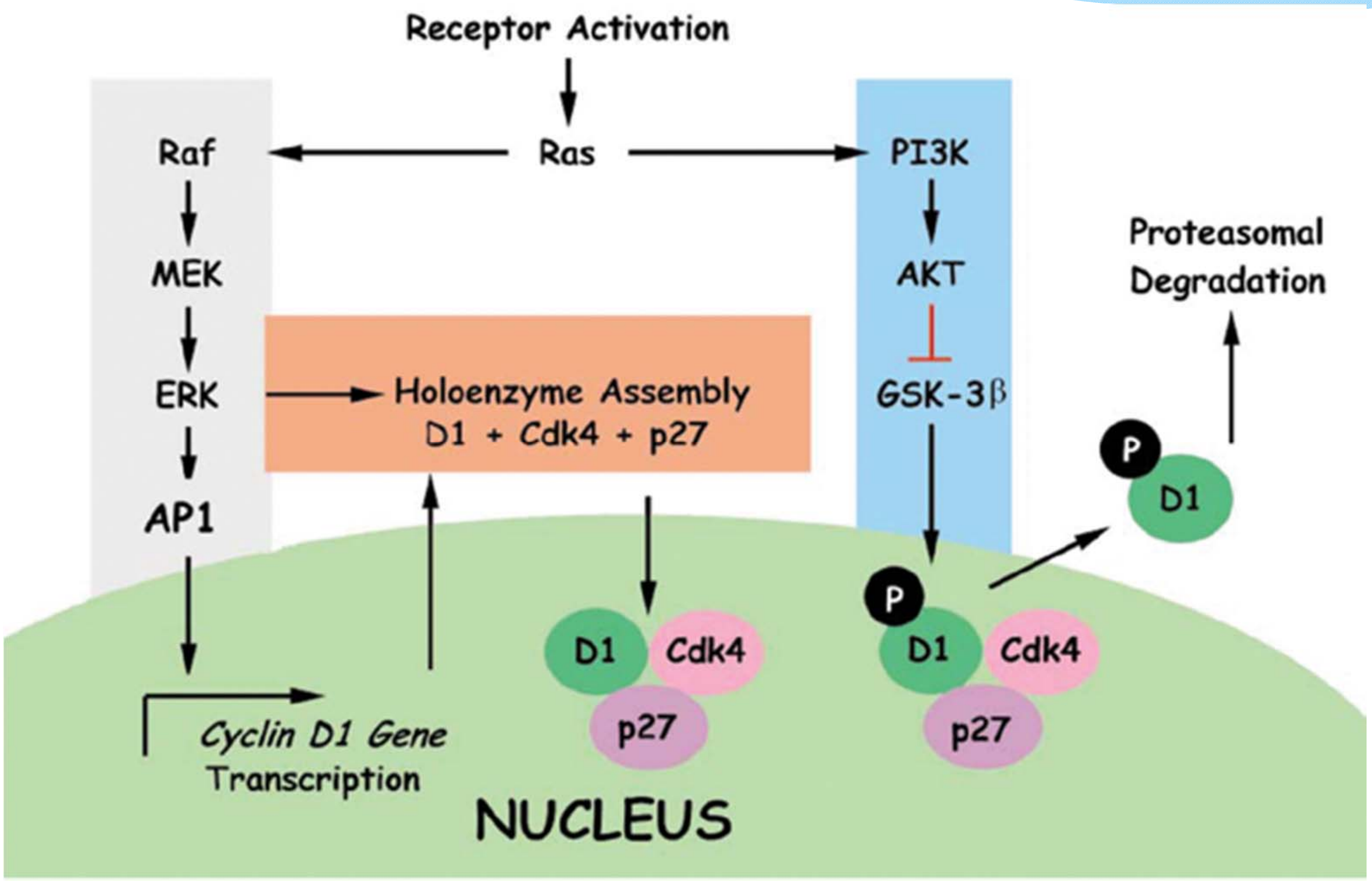
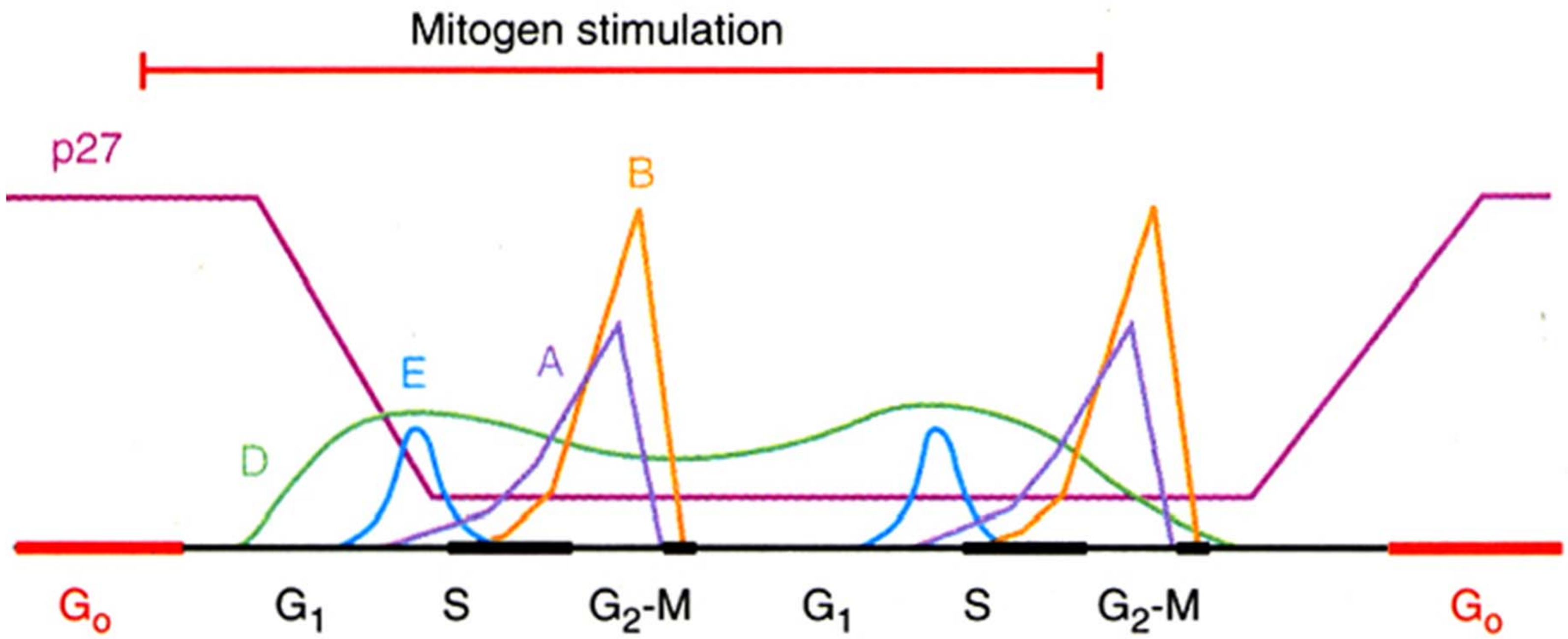
Growth Factors Induce Cyclin D1 Expression



Sherr and McCormick, Cancer Cell, Vol 2, 103-112 (2002)

Mitogen Induced Cell Cycle Progression in Cell Culture



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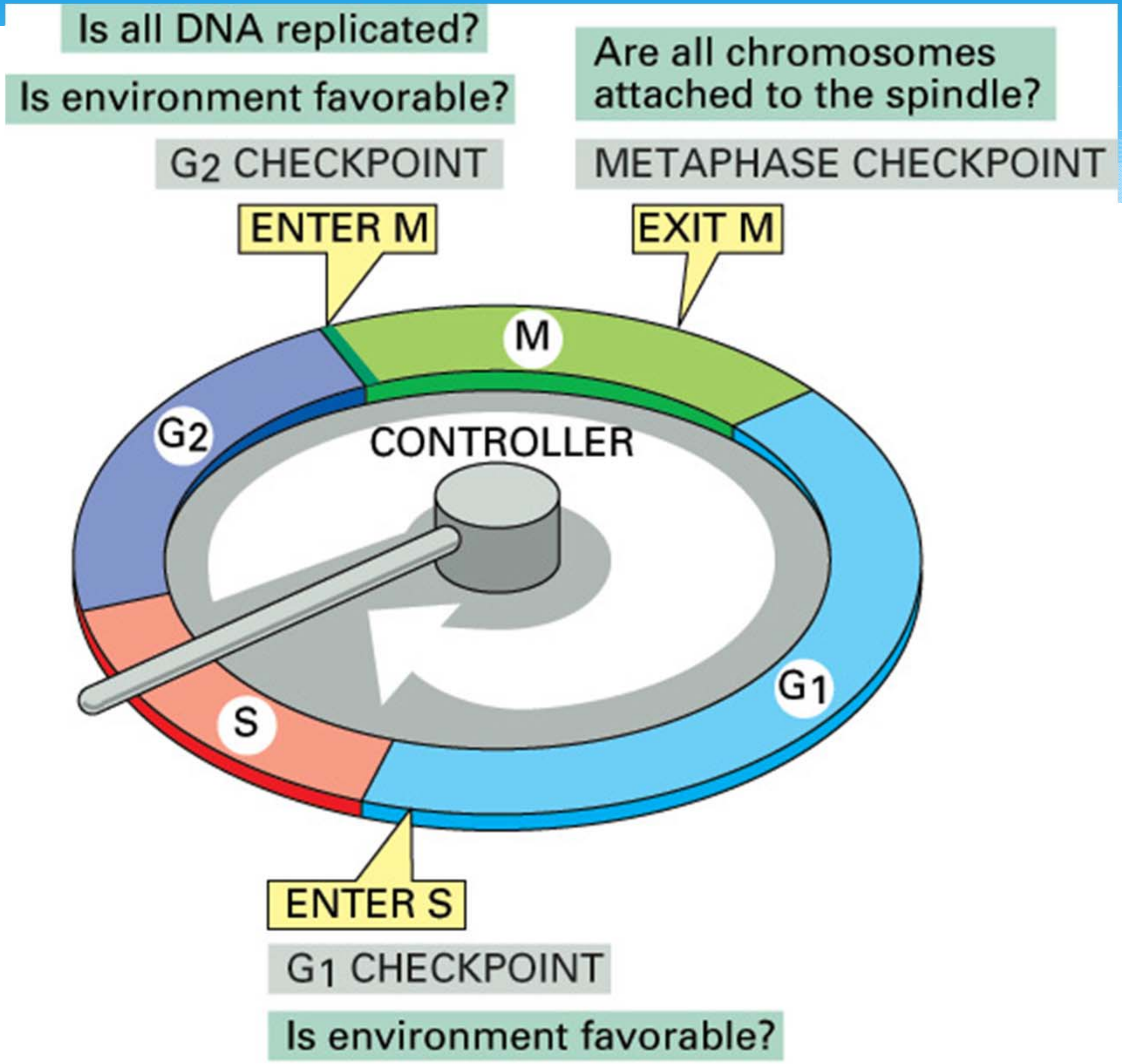


Figure 17–14. Molecular Biology of the Cell, 4th Edition.

Cell Cycle Checkpoints

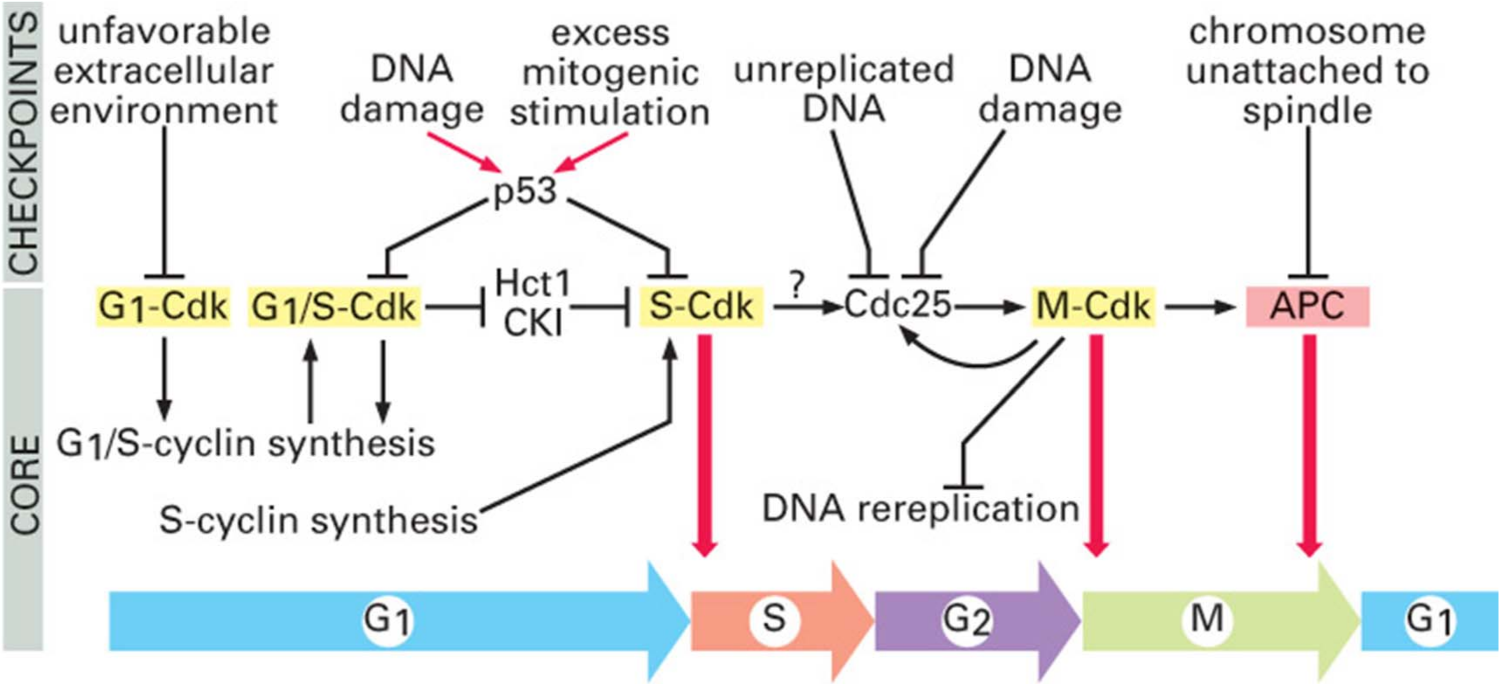


Figure 17–34. Molecular Biology of the Cell, 4th Edition.

Cell Cycle Checkpoints Improve Cell Viability

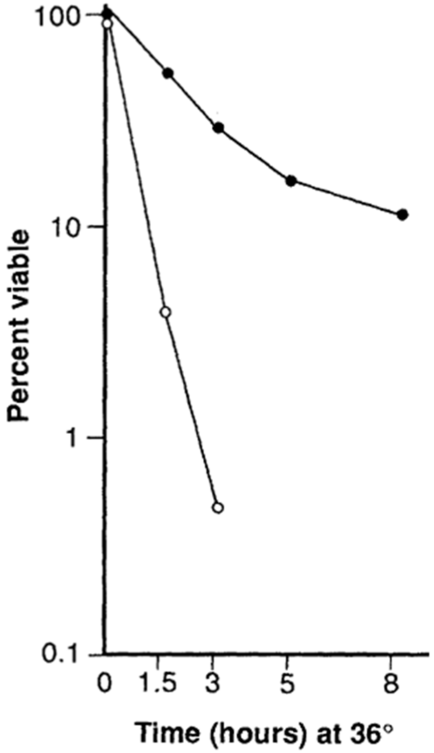
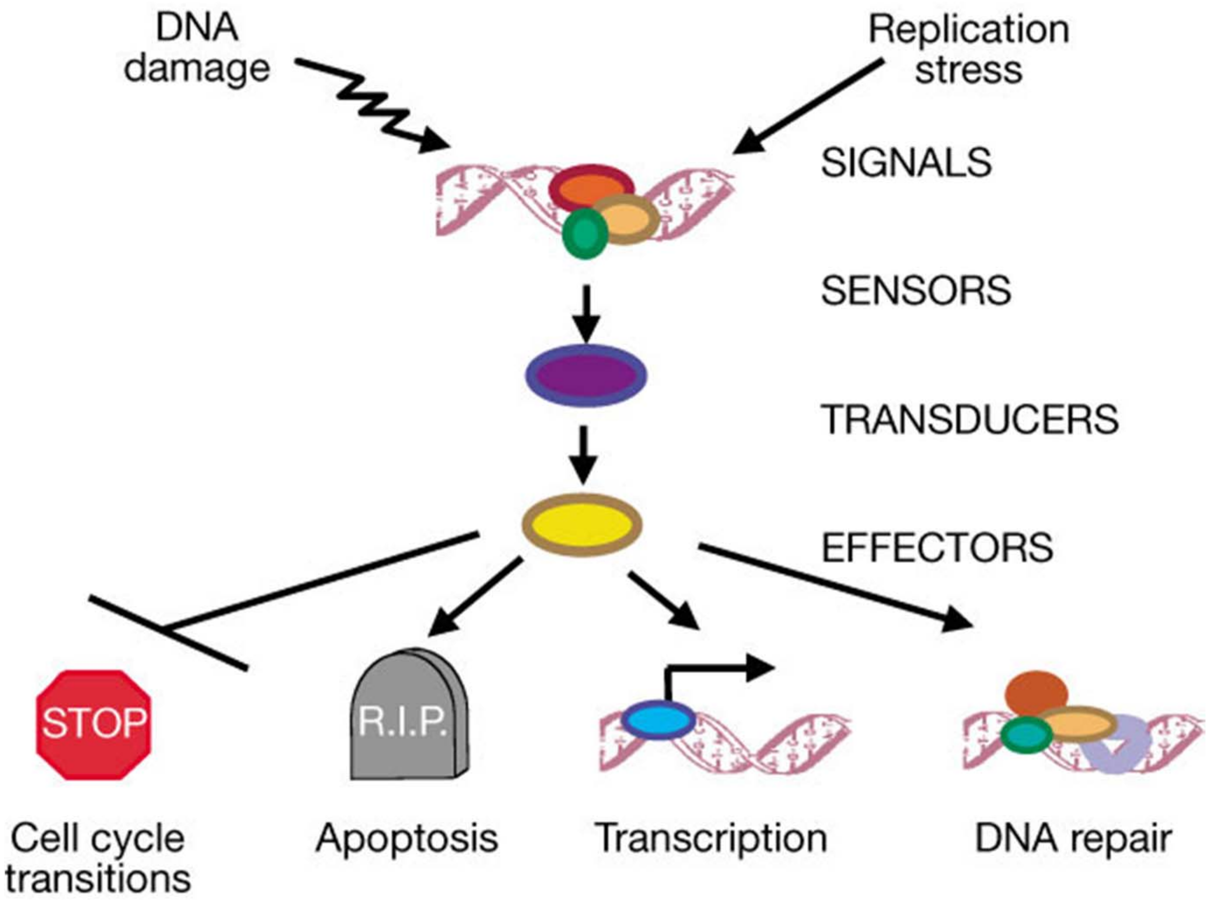


Fig. 3. Rapid loss of viability in cells defective both for DNA ligase and for the *RAD9* gene. *cdc9* (●) and *cdc9-rad9* (○) cells growing at 23°C were shifted to the restrictive temperature and viability was determined by plating for viable colonies at the permissive temperature (23°C). The cell viability reported is relative to viability at the time of temperature shift. Results were reproducible in separate experiments and with other congenic strains.

Weinert and Hartwell, Science 246:629 (1989)

How do Cell Cycle Checkpoints Work?



Zhou and Elledge *Nature* **408**, 433 - 439 (2000)

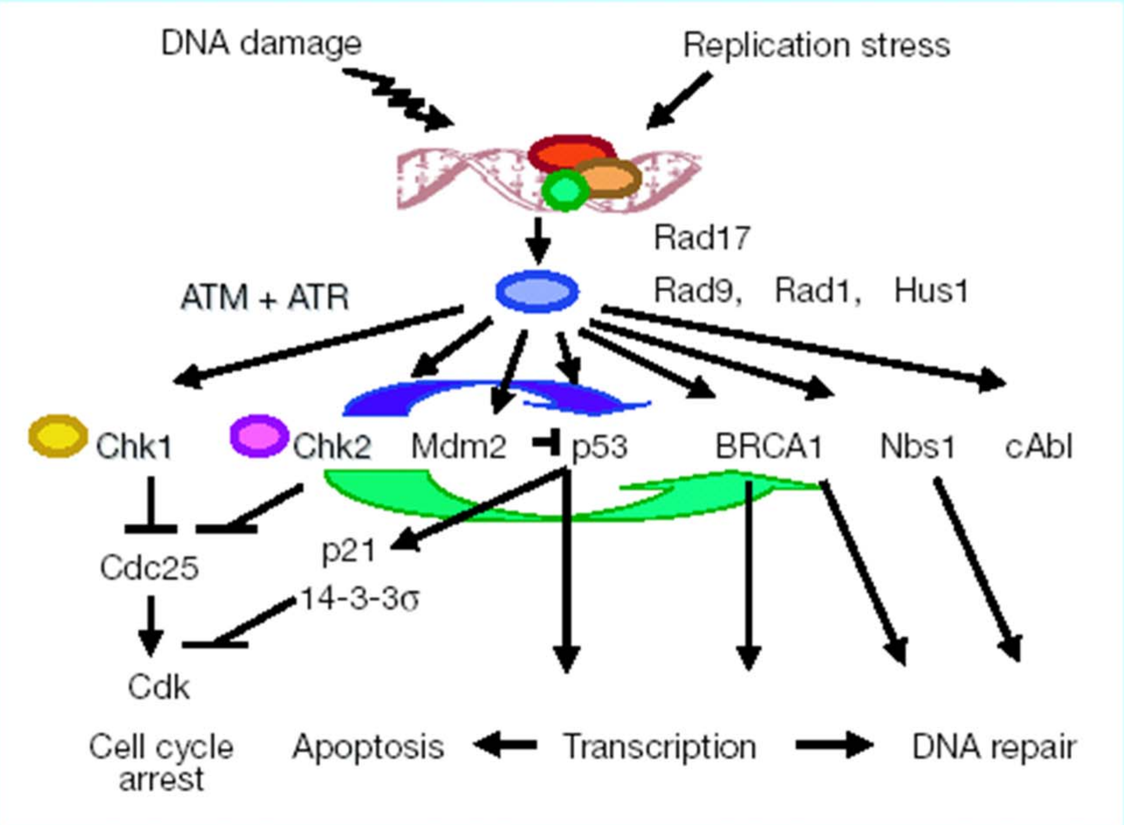
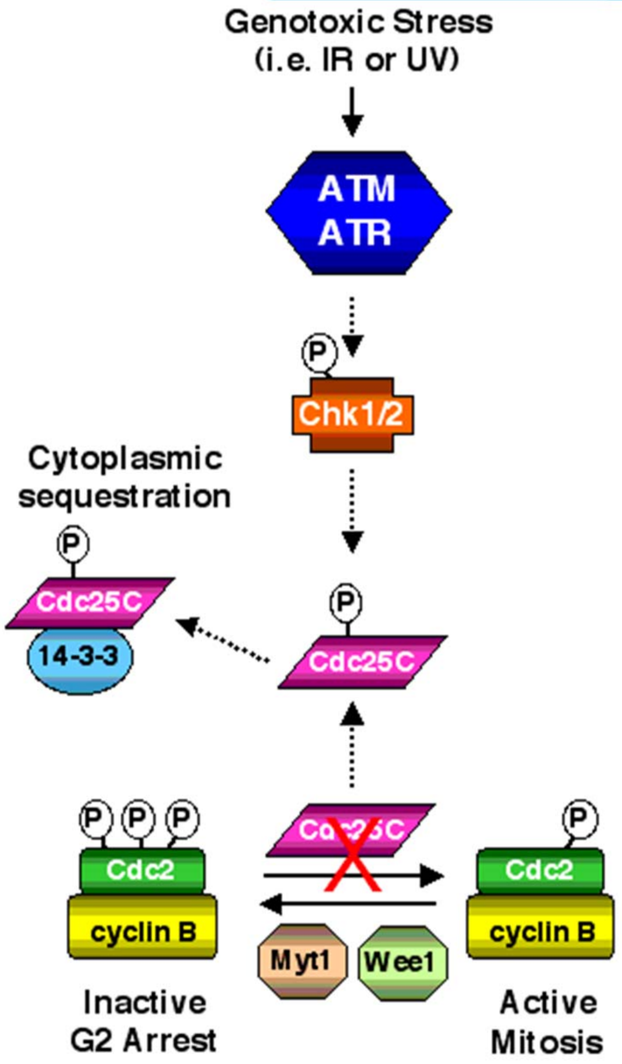


Figure 2 Organization of the mammalian DNA damage response pathway. Arrowheads represent positively acting steps while perpendicular ends represents inhibitory steps. Gene names are shown at the approximate positions where their encoded proteins function in the pathway. Although the general organization of the pathway is correct, some details are omitted, especially concerning the relationship between the ATR/ATM and Hus1/Rad17/Rad9/Rad1 proteins, which may participate in mutual regulation.

Zhou and Elledge *Nature* **408**, 433 - 439 (2000)



Signal

Sensor

Transducer

Effector

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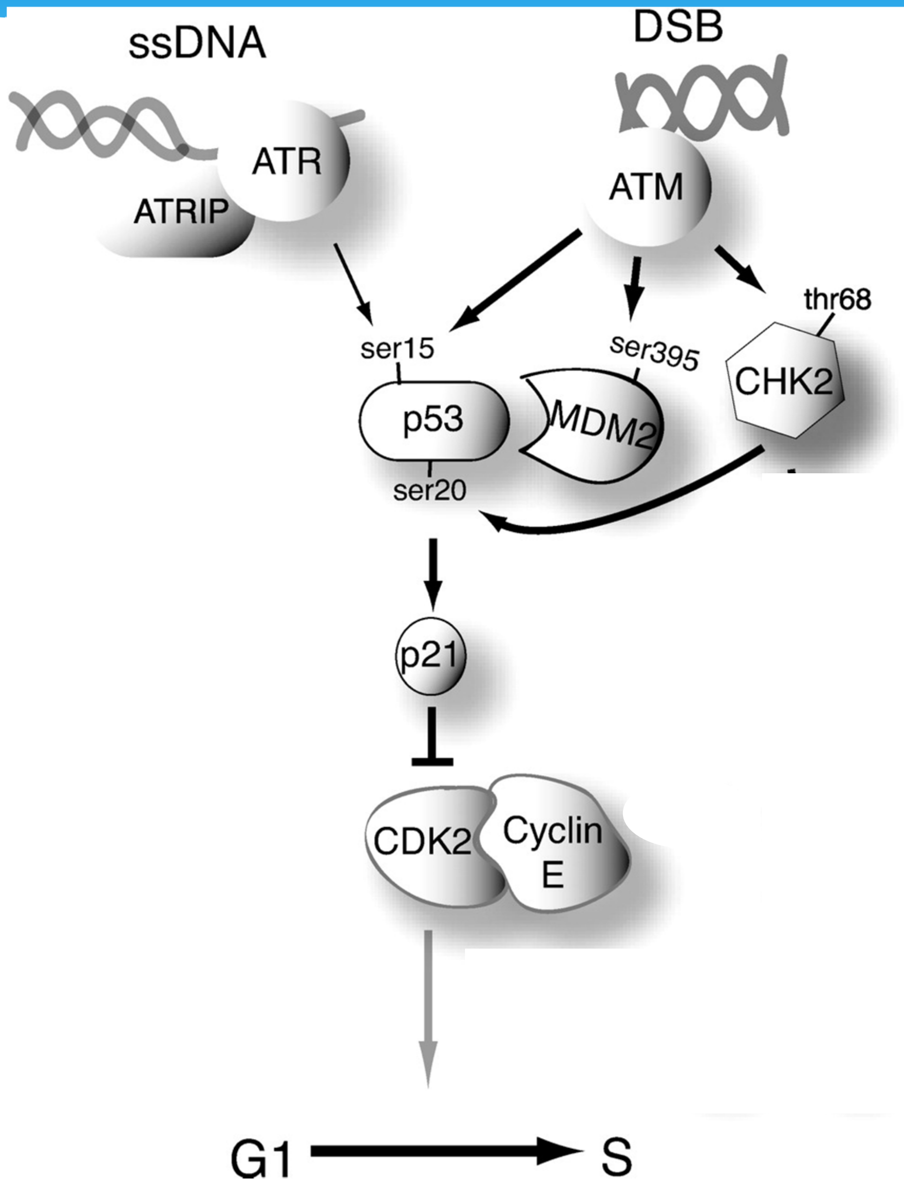

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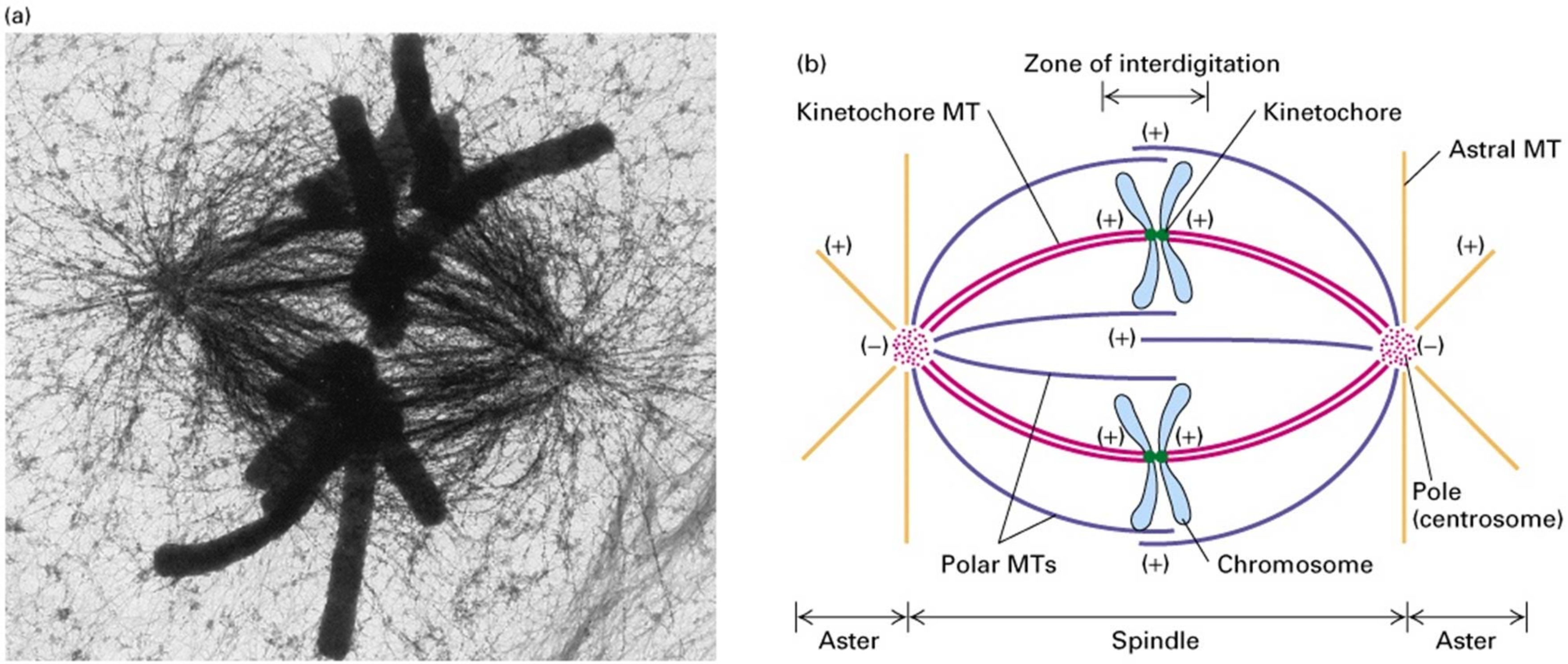
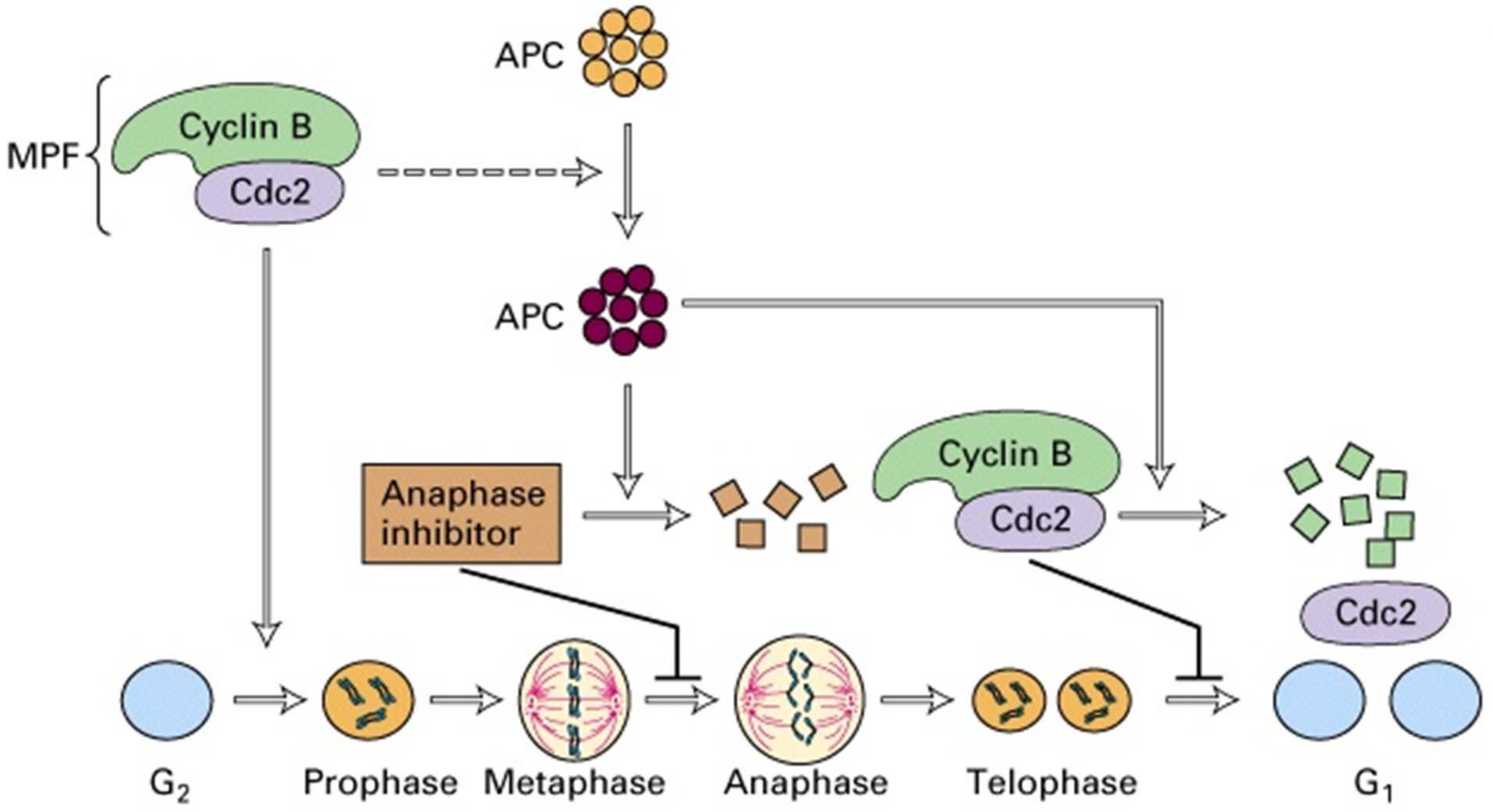
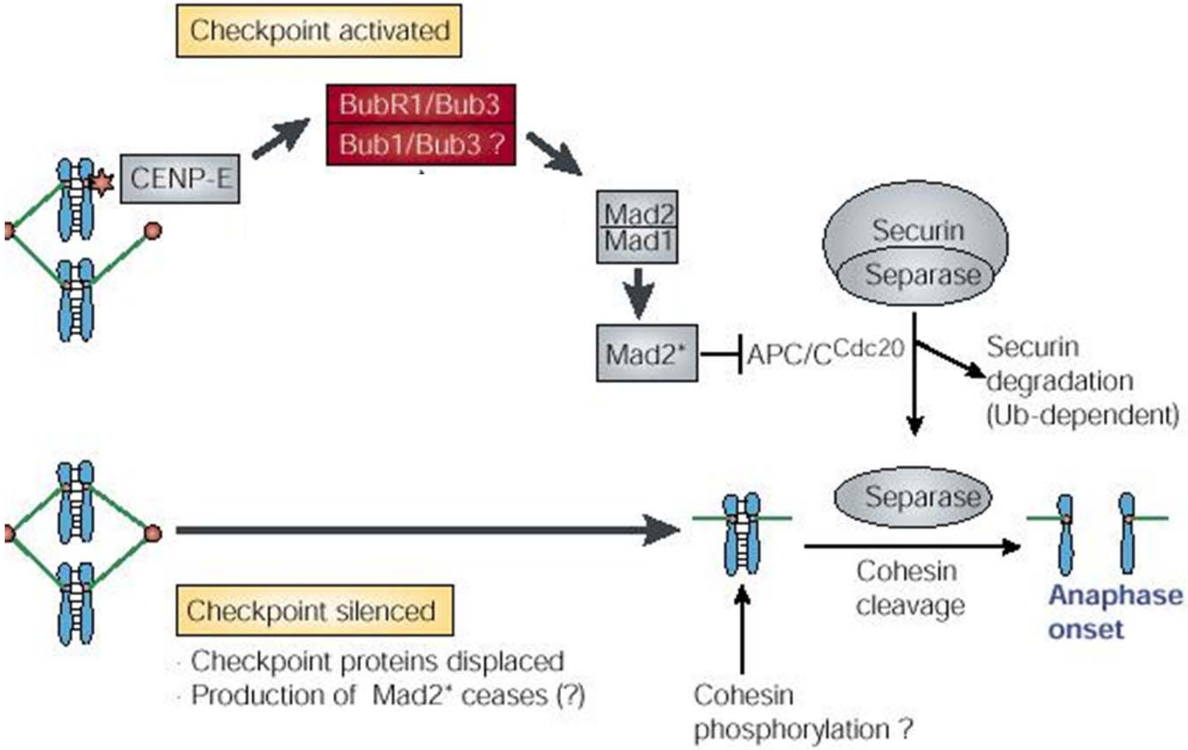
Metaphase in a mammalian cell



The Metaphase to Anaphase Transition



The Spindle Assembly Checkpoint



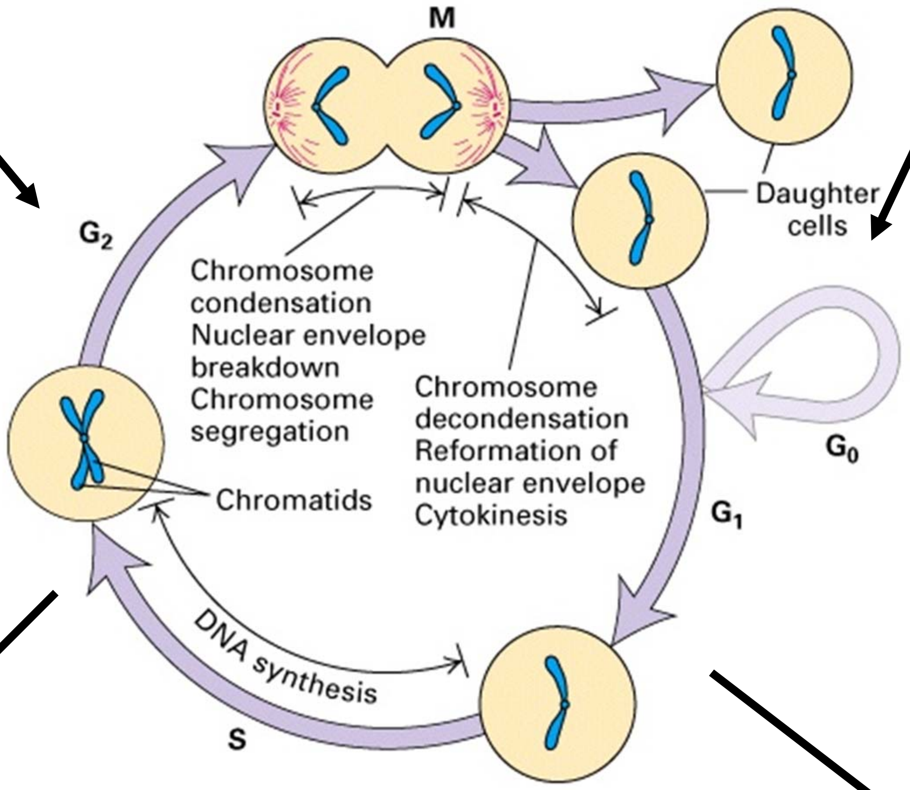
Checkpoint activated

Checkpoint silenced

- Checkpoint proteins displaced
- Production of Mad2* ceases (?)

poškození DNA
poruchy buň. cyklu
poruchy buň. dělení
nedostatek živin a růstových faktorů
kontaktní inhibice
vliv ECM

dostatek živin a růstových faktorů
mitogeny

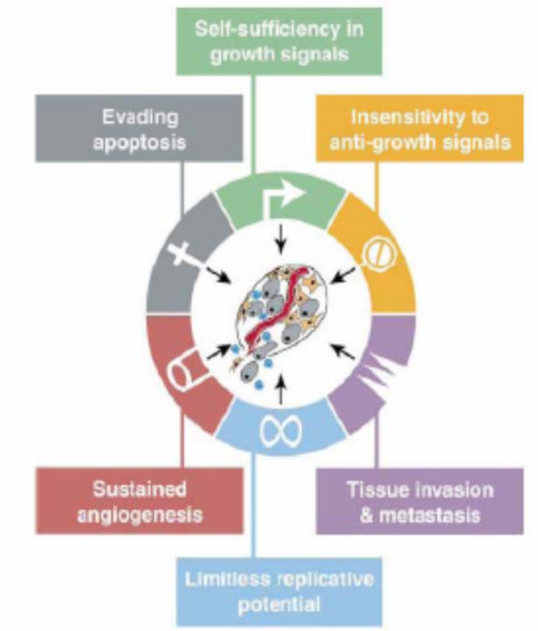


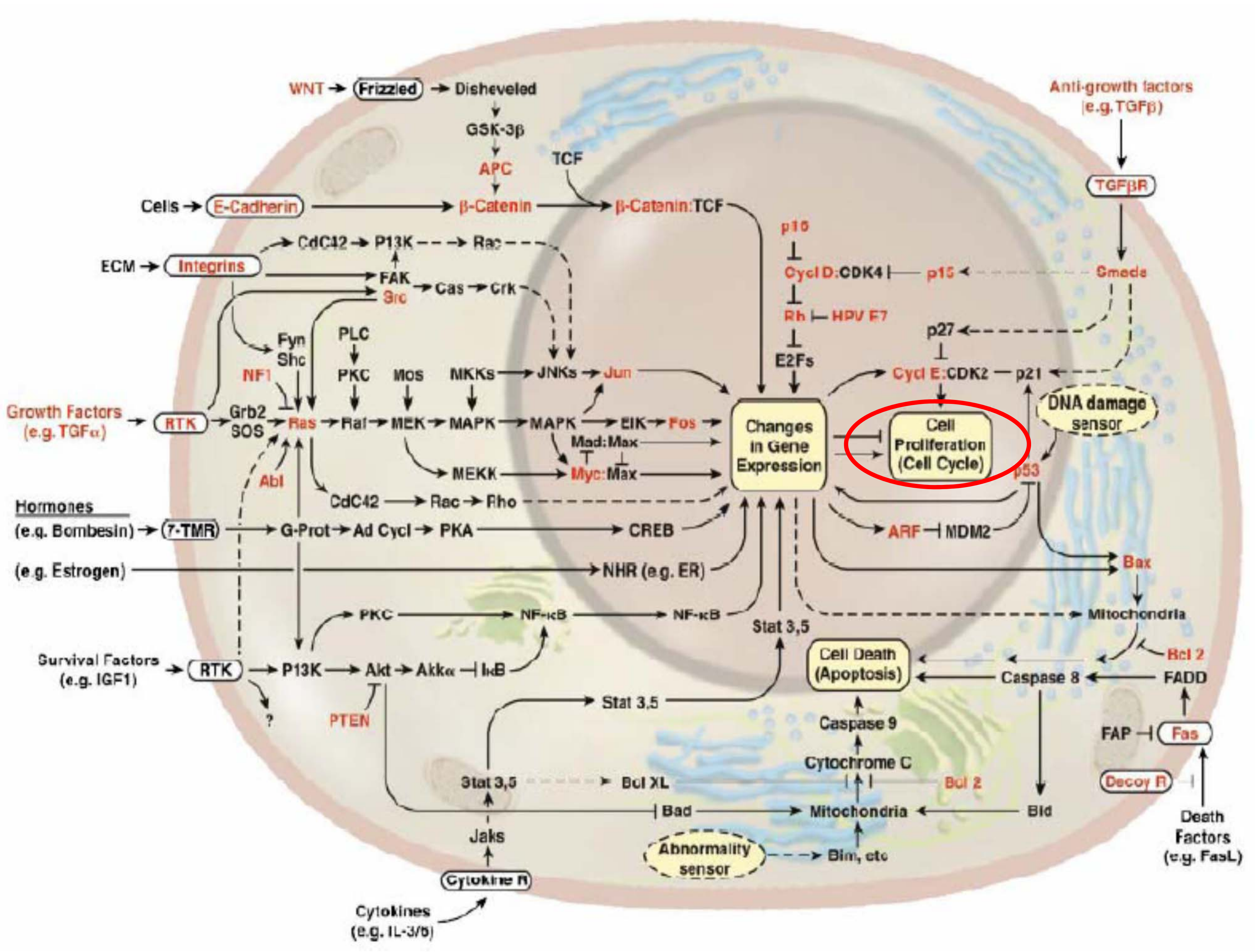
zástava buň.cyklu
(restriction point,
checkpoints)
setrvání v G_0 fázi
apoptóza

proliferace

Hallmarks of Cancer

- Summarized by Hanahan and Weinberg (2000) Cell
- Six changes for cancer – found in most, if not all, cancers
  - Self-sufficiency in growth signals
  - Insensitivity to growth-inhibitory signals
  - Evasion of apoptosis
  - Limitless replicative capacity
  - Sustained angiogenesis
  - Tissue invasion and metastasis




WNT
Frizzled
Disheveled
GSK-3β
APC
TCF
Cells
E-Cadherin
β-Catenin
β-Catenin:TCF
Anti-growth factors
(e.g. TGFβ)
TGFβR
ECM
Integrins
CdC42
P13K
Rac
FAK
Cas
Crk
Src
p16
Cycl D:CDK4
p15
Smads
Rb
HPV E7
E2Fs
p27
Fyn
Shc
PLC
PKC
Mos
MKKs
JNKs
Jun
NF1
Cycl E:CDK2
p21
Growth Factors
(e.g. TGFα)
RTK
Grb2
SOS
Ras
Raf
MEK
MAPK
MAPK
Elk
Fos
Changes in Gene Expression
Cell Proliferation (Cell Cycle)
DNA damage sensor
Mad:Max
Myc:Max
MEKK
Abl
p53
CdC42
Rac
Rho
Hormones
(e.g. Bombesin)
7-TMR
G-Prot
Ad Cycl
PKA
CREB
ARF
MDM2
(e.g. Estrogen)
NHR (e.g. ER)
Bax
PKC
NF-κB
NF-κB
Stat 3,5
Mitochondria
Survival Factors
(e.g. IGF1)
RTK
P13K
Akt
Akkα
IκB
Cell Death (Apoptosis)
Caspase 8
FADD
Bcl 2
?
PTEN
Stat 3,5
Caspase 9
FAP
Fas
Cytochrome C
Stat 3,5
Bcl XL
Bcl 2
Decoy R
Bad
Mitochondria
Bid
Jaks
Abnormality sensor
Bim, etc
Death Factors
(e.g. FasL)
Cytokine R
Cytokines
(e.g. IL-3/6)

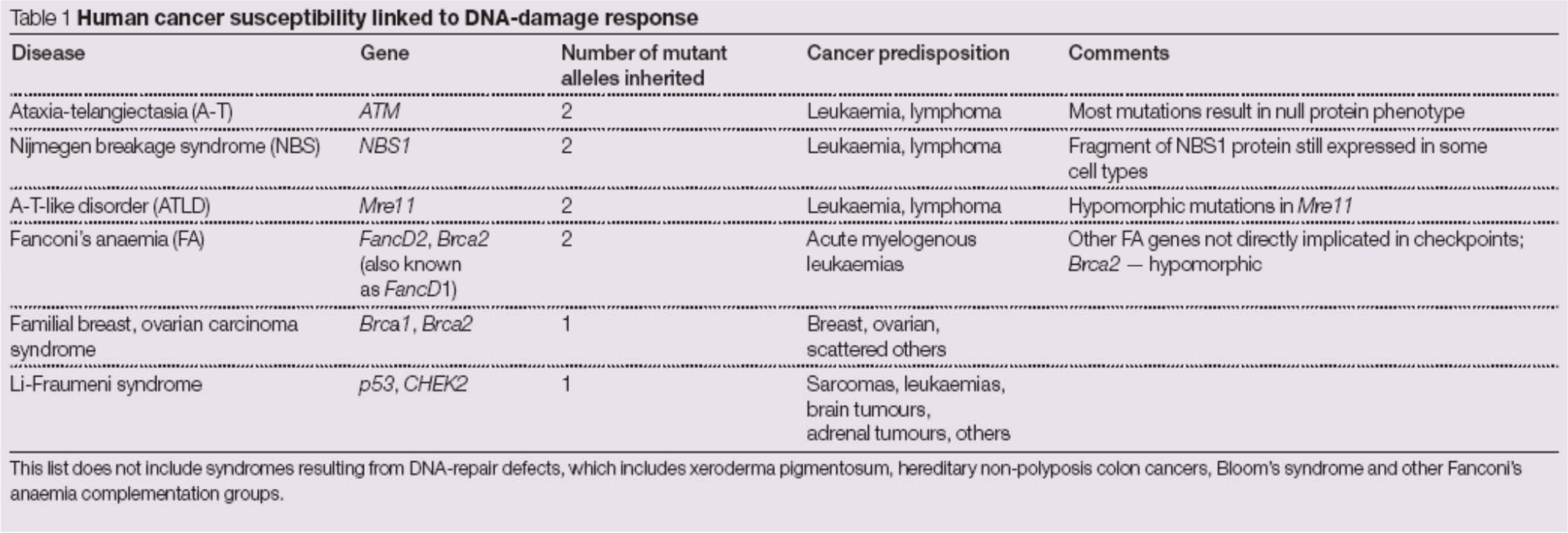


Table 1 **Human cancer susceptibility linked to DNA-damage response**

Disease	Gene	Number of mutant alleles inherited	Cancer predisposition	Comments
Ataxia-telangiectasia (A-T)	*ATM*	2	Leukaemia, lymphoma	Most mutations result in null protein phenotype
Nijmegen breakage syndrome (NBS)	*NBS1*	2	Leukaemia, lymphoma	Fragment of NBS1 protein still expressed in some cell types
A-T-like disorder (ATLD)	*Mre11*	2	Leukaemia, lymphoma	Hypomorphic mutations in *Mre11*
Fanconi's anaemia (FA)	*FancD2*, *Brca2* (also known as *FancD*1)	2	Acute myelogenous leukaemias	Other FA genes not directly implicated in checkpoints; *Brca2* — hypomorphic
Familial breast, ovarian carcinoma syndrome	*Brca1*, *Brca2*	1	Breast, ovarian, scattered others	
Li-Fraumeni syndrome	*p53*, *CHEK2*	1	Sarcomas, leukaemias, brain tumours, adrenal tumours, others	

This list does not include syndromes resulting from DNA-repair defects, which includes xeroderma pigmentosum, hereditary non-polyposis colon cancers, Bloom's syndrome and other Fanconi's anaemia complementation groups.